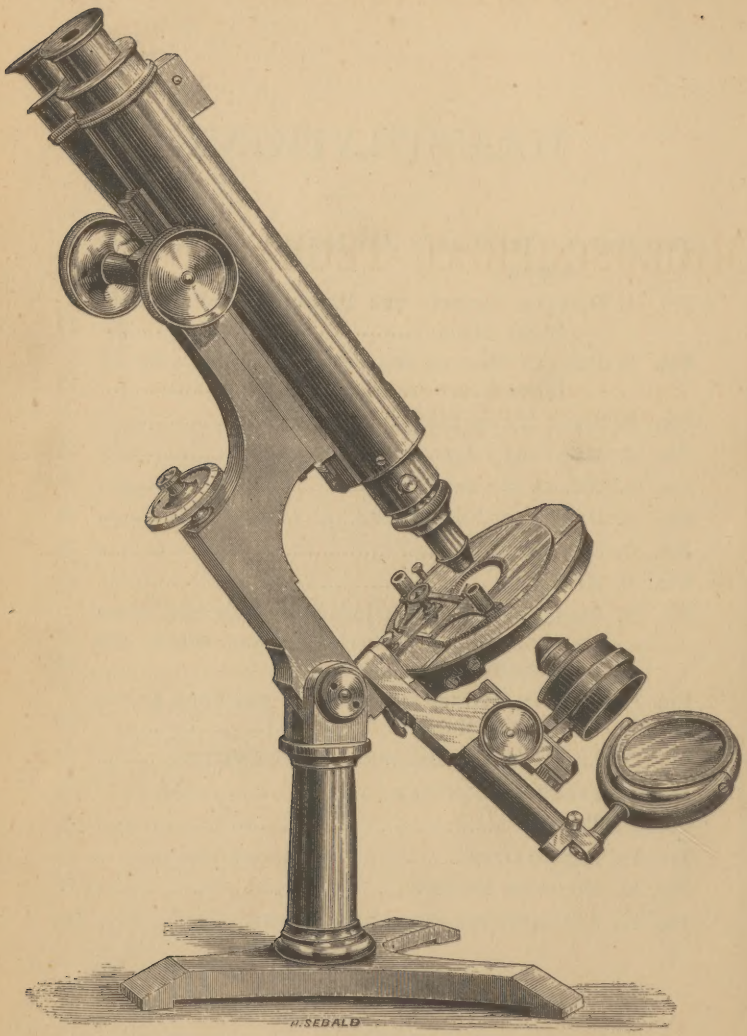
ILLUSTRATIONS.



ZENTMAYER'S NEW MODEL U.S.A.H. MICROSCOPE.

COMPENDIUM

OF

MICROSCOPICAL TECHNOLOGY.

A GUIDE TO PHYSICIANS AND STUDENTS IN THE USE OF THE MICROSCOPE AND IN THE PREPARATION OF HISTOLOGICAL AND PATHOLOGICAL SPECIMENS.

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PREFACE.

I was prompted to write this little compendium by the want, which I felt in my own studies, of a comprehensive treatise on the subject, which I could rely upon, and which would not mislead me by a large number of methods to be used in the preparation of tissues for microscopical examination. All the existing books on the subject, although they are excellent and indispensable to the advanced student, are too comprehensive, and are filled with the descriptions of methods of examination and preparation of objects which have not been thoroughly tried by the authors, and which are, therefore, frequently incomplete, by the omission of seemingly minor, but nevertheless essential, details.

In the present little volume it has been my plan, to which I have strictly adhered, to give a clear and short description of processes which I am in the habit of using myself, and which I have found to give uniformly satisfactory results. They may, it is true, not be the *best* in every case, but they will give results in the hands of the inexperienced which are sufficiently good to be serviceable, and as soon as the student is far enough advanced he will, without prompting, refer to

the larger books containing other methods, and will then be able to use them with advantage.

I have not thought it advisable to add a description of the histological details of tissues, because the field is one so extended that it would be impossible to do justice to the subject even in a volume many times larger than the present one; but I have added as an appendix a classification of the more common tumors and neoplasms, in tabular form, which I think will be of service to those engaged in the study of pathology.

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COMPENDIUM

OF

MICROSCOPICAL TECHNOLOGY.

CHAPTER I.

THE MICROSCOPE AND HOW TO USE IT.

The great number of instruments by different makers, and of different prices, often bewilders the student who wants to buy a microscope; and not knowing what will be most serviceable to him, he has to rely entirely upon the dealer for advice, which, to say the least, is always risky. A short description of what is indispensable in an instrument for histological work will, therefore, not be out of place.

The Stand should be of such construction and weight that no matter in what position the body of the microscope may be it will be firm. In practice it has been found that the tripod base is the best in this respect.

The Body, consisting of the tube with the ocular and objective, the stage, the sub-stage and the mirror, should be mounted upon the base in such a manner that it can be inclined at any angle, from the upright position to the horizontal, and there should be a *stop*, which prevents further inclination beyond either the upright or horizontal position. This, although seemingly trivial, is of

great importance, as it will be found impossible to make protracted microscopical observations with the body of the observer in a constrained position, which he must assume if his instrument cannot be inclined. Furthermore it is necessary that the body of the instrument should be absolutely horizontal in making drawings or measurements with the camera lucida, which position cannot be obtained without a stop that will not allow the inclination to go any further.

The Stage should be either a mechanical one—that is one which can be moved, by means of rack and pinion, sideways or up and down—or what is called a hand-stage, which consists of a piece of glass held on the stationary stage by means of a spring and ivory-pointed screw. This arrangement allows the stage and the object to be moved in any direction and at any inclination. The two clips for holding the object, which are found on many instruments, are decidedly objectionable, because they are very apt to get in the way and injure the specimen. On such microscopes a very good movable hand-stage may be improvised by removing the clips with their posts from the stationary stage-plate, so that an even surface is obtained; a piece of plate glass about one-sixteenth of an inch thick and a little larger square than the stage-plate, with a round hole one inch in diameter cut in its middle, is then agglutinated, by means of a little glycerine, to the stage-plate and a ledge of glass or metal, or even pasteboard, is cemented on to the lower edge of the glass plate, upon which the slide may rest. It will be found that the atmospheric pressure is sufficient to hold this glass stage firmly at all angles

of inclination and at the same time allows of free motion in all directions. The glycerine should be renewed from time to time, and the glass as well as the permanent stage should be kept clean, to insure easy motion. A very great advantage, especially in pathological investigations, is to have the greatest possible amount of motion of the stage, as, otherwise, large sections cannot be satisfactorily examined; and there is no doubt in the minds of those who work much in histology and pathology that very soon the makers of microscopes will be compelled to give us instruments with stages which have three or four inches motion either way.

The Sub-stage and Mirror should be so arranged that they can be adjusted to the proper distance from the object, and that they should be *absolutely* centered. The bar upon which the mirror is mounted is usually hinged to the stage or limb of the instrument, so that oblique illumination may be obtained; it should, however, be provided with a spring stop which indicates when the mirror bar is in a line with the optical axis of the objective.

Most of the cheaper instruments are not provided with a sub-stage condenser, nor even with a sub-stage, and the owner of such a microscope must use his ingenuity in improvising a condenser, which, for histological work, is almost indispensable. The method of using this part of the instrument will be considered in the section on illumination, but it will not be out of place here to say that *a sub-stage condenser which is not absolutely centered is worse than none at all.*

The Tube should be either ten inches long, measured

from the objective to the ocular, or should have within it a draw-tube which may be drawn out so as to make the whole ten inches long. It should be mounted upon the body by means of a rack and pinion slide, called the coarse adjustment, which latter should work smoothly and without jarring, and the instrument should be provided with a fine adjustment. In the European instruments this is usually attached to the tube itself, while in the American microscopes it forms part of the body, which latter arrangement is to be preferred, because the tube is not changed in length when working the fine adjustment. In examining an instrument notice should be taken whether in moving the fine adjustment screw the field moves, and if so the microscope should be rejected as unfit for good work. There is a doubt in many minds whether a binocular microscope is better than a monocular one for histological work, and the difference in cost prevents many a one from purchasing a binocular microscope. It will, however, be found that a binocular instrument is more serviceable in the greater number of cases than a monocular, because it enables the observer to keep both eyes open, which is a great saving to the organs, and it gives him, with low powers at least, the benefit of stereoscopic vision; then again, if it be of the Wenham form—the best and most generally used—it can readily be changed into a monocular instrument when occasion requires it.

Oculars or eye-pieces are always furnished with the instruments, and are usually of good quality. They are either lettered or numbered (A, B, C or 1, 2, 3), thus indicating their magnifying power. For ordinary work

two eye-pieces, A and B, are sufficient, and only where more amplification is absolutely necessary, are the higher oculars of use.

Objectives. The choice of objectives is beset with great difficulties for the beginner in microscopy, and it is as difficult to give any advice in the matter in mere words; a few hints, however, will perhaps help the student in picking out good objectives.

In the first place, the powers best suited for histological studies are a one inch or two-thirds, a one-fourth or one-fifth, and if desired, a one-eighth or one-tenth objective.* Secondly, they should be free from aberration.

In order to test objectives, both for chromatic and spherical aberration, place a few minute globules of mercury upon a dark background slide and view them as opaque objects, by artificial light. If the image of the flame reflected from the surface of the globules (focusing the objective in and out) appears as a sharply defined circle of light, the lens is corrected for spherical aberration, and if the disk of light is free from color, for chromatic aberration also.

The lenses should also have a flat field, which may be determined by examining a layer of blood corpuscles upon a glass slip prepared in a manner which will be described hereafter. If after carefully focusing the disks near the edge of the field are as distinct as in the centre, the lens is a good one as regards flatness of field.

Finally, the objectives should possess a moderately

* These fractions designate that the system of lenses comprising the objective is equivalent, in focal length as well as magnifying power, to a single lens of the same denomination.

high angular aperture, because their working distance is large, thus allowing the use of thicker cover glasses, for the objects are therefore more easily manipulated, and they are not as expensive as the very wide-angle objectives. On the whole, the student in picking out lenses is certain of getting good objectives by depending upon the reputation of the maker; but it is an undeniable fact that different opticians produce different powers better than all other lenses of their make, which renders it somewhat difficult to get the best in the market. The so-called French lenses, which are sold at a very low figure, are the only ones which are absolutely useless.

ILLUMINATION.

We will suppose, now, that we have a good microscope before us, and that we want to use it for the examination of an object. The first thing we have to do is to arrange our light so that we may get the best possible result from our optical combination of ocular and objective. Let us remind the reader, here, that often more depends upon proper illumination than upon the quality of objectives, and that the injury to the eyes due to microscopical studies, which is so often complained of, is almost entirely caused by a want of proper care in the illumination of the object. Artificial light, such as is obtained from a good kerosene lamp, is to be preferred to daylight, for the simple reason that the former can be controlled as to its brilliancy, while the latter is beyond our control.

In arranging our illuminating apparatus we must have something to judge of the quality of the light as it

passes through the microscope, and it will be found that there is no better test than a slide of blood, prepared in the following manner: Take a clean one-by-three glass slide, and place near one end of it a drop of fresh blood, obtained from the prick of a needle in the finger. Then take another slide, with a ground edge, and place its edge into the drop of blood, inclining the second slide until it stands at an angle of about forty-five degrees toward the first one, and draw it quickly but evenly across the first slide (FIG. 1). The result will be that

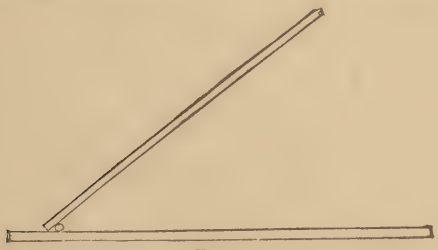

FIG. 1.

the blood corpuscles are spread evenly upon the slide, in *one* layer only, thus giving an excellent view of their outline.* The blood corpuscles being lenticular bodies, with depressed centres, act like so many little lenses of glass, and show defraction rings, if the light is not properly arranged. It will, therefore, be seen that a slide prepared in this manner forms one of the best, if not *the* best, tests for illumination, as well as for flatness of field.

Having placed the test slide upon the stage of the

* I am indebted to Dr. J. J. Woodward for this excellent plan of making an even blood slide.

microscope, and using a low-power objective, the lamp is next arranged on the left-hand side, in such a position that the flame presents a three-fourths profile view to the mirror, and is a little in advance and below the stage of the instrument when the latter has been placed in a convenient position. A bull's-eye condenser is then placed in front of the lamp and so arranged that the concave mirror is in the centre of the circle of light as it is seen upon a piece of white paper held behind the mirror. The latter is then so turned as to throw the light up through the condenser, thus illuminating the field. In order to neutralize the yellow color of the lamp light, which is very fatiguing to the eye, and which reduces the brilliancy of the staining of objects, a piece of blue glass should be attached to the lower opening of the sub-stage, through which the light from the mirror has to pass.

The sub-stage condenser is then to be accurately centered, if it has a centering attachment, which may be done by moving it up close to the slide and focusing upon it with a low-power lens. If out of centre it will be found that the circle of light is either to one side or the other of the field, and it should be made to fall exactly in the centre. When thus centered the one-fifth objective is used; if the student possesses a nose-piece*

* A nose-piece is an attachment to the end of the tube of the microscope, by means of which two or more objectives may be brought into proper position, one after the other, by simply revolving the swivel to which the objectives are attached. It acts somewhat like the drum in a revolver, which, by rotating, brings the different cartridges successively in the proper position to be fired out of the barrel.

this change will entail no loss of time; the condenser is moved down until the image appears clear and brilliantly lighted. When in the right position the light from the condenser is frequently too bright to be borne by the eye with comfort for a length of time, and it should then be toned down, by means of a little perforated cap or diaphragm, which is placed over the front lens of the condenser, or below and close to the system of lenses. When thus illuminated the blood corpuscles should appear as slightly olive-colored disks, with a fine but intensely black outline, and on changing the focus there should appear a spot in the centre. In order to fully appreciate the importance of each one of the parts of the illuminating apparatus and the necessity of having them in their proper position, let the student first remove the bull's-eye and let the light of the lamp alone fall upon the concave mirror, without change in anything else. He will then see that the outline of the blood disks is much less sharply defined, and that there is a suspicion of another outline within the outer one. Let him then move the mirror bar slightly to the right or left of the median line, and he will find that this second outline will be more marked and a third one will be faintly seen, while the true margin of the corpuscle is far from being sharp. Let him, finally, remove the sub-stage condenser from its proper position, or throw it out of centre, or take it off altogether, and he will find the blood disks filled with rings and with a bright spot in the centre (Fig. 2).

Opaque objects, such as are too thick to be made transparent, or are mounted upon a slide with a dark back-

ground, must be lighted from above, which may be accomplished in the following manner: Place the lamp to the left of the stage and raise it so that its flame is several inches above the stage of the instrument, when the latter is in a position convenient for the observer. Then place a small bull's-eye condenser between the stage and lamp, but close to the object, and turn it so that the light is concentrated upon the object to be viewed. In some microscopes of American make the mirror bar is attached to the limb of the instrument at a point which is in a line with the object on the stage, so that the mirror can be swung around and above the

FIG. 2.

stage without altering its relative distance from the object. In such a microscope opaque objects can be illuminated without the use of a bull's-eye lens, by simply reflecting the light of the lamp upon the object with the concave mirror swung above the stage and as close to the tube of the instrument as possible. In this case the lamp should be in front and a little below the stage.

Still another method is by a parabolic reflector attached to the objective, which collects the light and concentrates it upon the object. This can, however, only be used with very low powers having long working distances.

The Camera Lucida is a piece of apparatus used in measuring and drawing objects under the microscope. It consists, in its best form, of a prism of glass attached to the ocular in such a manner that the image formed by the optical combination of objective and ocular is thrown into the eye of the observer, and the image appears, in the same direction that the rays of light strike the eye, as if reflected upon a piece of white paper placed upon the table, upon which its outlines may be traced with a pencil. The same may be accomplished by placing a slightly tinted piece of glass, known as Beale's neutral-glass camera lucida, held at an angle of forty-five degrees to the axis of the instrument, close to the eye-lens of the ocular, which, however, does not give as brightly illuminated an image, and on account of the double reflection, is not suitable for drawing details. Such a reflector, answering the purpose admirably, may be improvised by attaching a thin cover-glass at an angle of forty-five degrees to the cap of the ocular, by means of a small piece of beeswax. In order to use either the prism or the reflector the instrument is to be inclined until its tube is horizontal, a piece of white paper, which should be shaded from all extraneous light, is placed upon the table, directly underneath the ocular, and the *left* eye is placed so, above and close to the camera lucida, that in looking downward the image appears to be upon the paper. Keeping the right eye open at the same time, the point of the pencil tracing the outline of the objects upon the paper can be seen, and thus the motion of the hand holding the pencil can be regulated. In order to obtain an image upon the

paper of the same size as that seen in the microscope, the distances from the centre of the eye-lens of the ocular to the surface of the paper should be the same as from the ocular to the objective, viz., ten inches.

MEASURING.

The apparent size of objects seen in the microscope is of great importance in their recognition, and as this varies with the magnification employed in viewing them, it is necessary for the student to know the magnifying power of his objectives and oculars in their different combinations. The magnifying power of an optical combination of objective and ocular is expressed by saying that it magnifies so many diameters, which means that an object seen—a blood corpuscle, for instance—has its diameter magnified so many times. In order to obtain the magnification of the surface of an object, the number of diameters should be squared, and such surface magnification is expressed by saying that an object is magnified so many *times*, an expression not used in microscopy, but utilized by unscrupulous dealers to mislead purchasers.

In order to find the magnifying power of the optical combination, the instrument is placed horizontally, the camera lucida is attached to the ocular, and a piece of paper is placed on the table ten inches from the centre of the eye lens, and shaded from extraneous light. A stage micrometer (Fig. 3.), which is a slip of glass upon which lines have been ruled one-hundredth and one-thousandth of an inch apart, is placed upon the stage and the image of the lines carefully focused upon the paper. With a

sharp-pointed pencil, the lines are then marked upon the paper and the distance of the dots is measured carefully with a pair of compasses and an inch rule; preferably divided into tenths of inches. It will thus be seen that if the distance between two lines which on the stage micrometer are one-hundredth of an inch apart measures upon the paper five-tenths of an inch, the magnification is fifty diameters. The student should make himself a table giving the different powers of his objectives in connection with the oculars as found in the manner described above, which he will find very convenient for reference. Objects, such as small animalcules

Fig. 3.

or histological elements, may be measured in a similar manner, viz: By drawing their outline with the camera lucida, and then measuring the diameter with a pair of compasses, then, knowing the magnifying power of the optical combination, a simple calculation will give the size of the object. To exemplify this, let us say that the objective and ocular give a magnification of fifty diameters, and the diameter of the object measures five-tenths of an inch as seen with that power; its actual size will then be one-hundredth of an inch.

An easier and more simple method of measuring objects is with the *eye-piece micrometer*, also a slip of

glass with fine lines ruled upon it, which is introduced into the ocular between the two lenses. The distance between the lines of the eye-piece micrometer is not known, and varies with the different powers used. In order to measure an object under the microscope with this micrometer, the object upon the stage is moved until the lines fall directly on its margin, and the number of intervals the object fills is noted down. The object is then removed from the stage and the stage micrometer substituted for it, which, in turn, is moved until its lines coincide with those of the eye-piece micrometer, and it is noted how many intervals between the lines it takes to fill the intervals of the eye-piece micrometer, previously noted down as having been filled by the object to be measured. The distances between the lines of the stage micrometer being known, the number of intervals noted express the size of the object. For instance, we wish to measure an object which exactly covers three intervals of the eye-piece micrometer, and find, on substituting the stage micrometer, that two intervals of its lines ruled one-hundredth of an inch apart cover three intervals of the eye-piece micrometer; we then know that the object is one-fiftieth of an inch in diameter.

Drawing and measuring with the camera lucida or reflector requires some skill and practice, and the student should, therefore, not place too much reliance upon his first attempts, but should repeat the measurements determining the magnifying power of his lenses over and over again, until the results of many trials coincide, before he looks upon them as correct.

Under this head it will be necessary to describe

another very useful accessory to the microscope, and one which will save the possessor a great deal of time if properly and systematically used. This is a Maltwood's finder, by means of which a particular spot or place in a specimen can readily be found again. It consists of a slide of glass which has photographed upon it a large number of minute squares which are numbered in a certain system. See Figure 4. If, now, a place in a

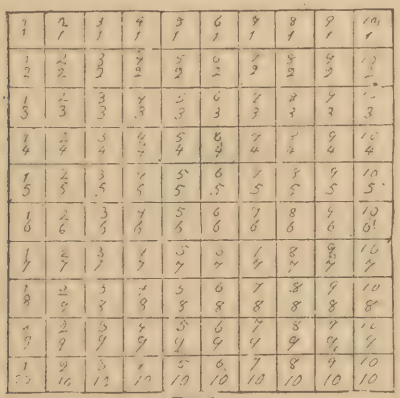

Fig. 4.

specimen is found which we wish to be able to find again without loss of time—for instance, a trichina spiralis in a specimen of muscular fibre—the object is removed from the stage and the finder is substituted for it, taking care to have it rest against the stop on the ledge of the stage. By looking through the microscope the numbered squares are seen, for instance, $\frac{5}{3}\frac{6}{3}$, and these numbers are then recorded upon the label of the specimen. If we now wish to find a place thus marked on a slide, we

place the finder on the stage and move the latter until the numbers recorded on the label of the slide are seen in the field; we then substitute the slide for the finder, and the spot which we desired to find will be in the field of the instrument. Some instruments of American make, with mechanical stage, have the moving parts divided into degrees and thus may be used as finders, for, by observing and recording the number of degrees of lateral as well as of vertical displacement of the stage, the same spot in a specimen may always be found again by displacing the stage to the same number of degrees as recorded.

This method is, however, only applicable to instruments by the same maker, and, if we wish to send a specimen to a friend at a distance who does not own such an instrument, he cannot find the spot designated. If we use, however, Maltwood's finder, anybody who possesses this accessory can, on any instrument, find the place indicated on the label without any trouble and without loss of time.

CHAPTER II.

PREPARATION OF ANIMAL TISSUES.

All objects must be prepared in some way before they can be satisfactorily examined under the microscope. Some objects, such as the wings of insects, require but little preparation, while others, such as histological and pathological tissues, require considerable manipulation before they can be studied and their details demonstrated. As this latter class of tissues is more difficult to prepare than, perhaps, any other, and as it is of greater importance to most persons using the microscope for investigation, it will be considered first. But before entering into a detailed description of the different processes employed, it is necessary to say a few words in regard to the general method the student should follow in order to be successful as a preparer of microscopical specimens.

First, he should *strictly* adhere to the formulæ given, and should not omit even seemingly insignificant details, or even try to improve upon them before he is thoroughly master of the process.

Secondly, he should be content, at first, with one process, and should not try another and another because of failure, for although particular pains will be taken in describing *all* the details of a process, yet the necessary skill cannot be imparted to the student in writing. He should, therefore, try again and again before condemning a formula as useless.

Thirdly, he should endeavor to trace the cause of his

failures, and thus learn by them; he will then more quickly master the matter in hand. And *finally*, he should preserve scrupulous cleanliness in his laboratory or work-room, and should be methodical as regards time and saving as regards material.

If every worker in microscopy would bear these rules in mind, less money would be spent for material, fewer often valuable specimens would be spoiled through want of proper attention, and better preparations would be found in cabinets.

TEASING.

Any tissue, in order to be fit for examination, must be made transparent, so that its histological elements may be studied by transmitted light. There is, however, an exception to this rule, when only the surface of a tissue is to be examined and viewed as an opaque object. In order to make tissues transparent they must be made thin and must then be immersed in some substance which will transmit light readily, and will at the same time permeate the tissue, very much in the same manner as paper is made translucent by oil. This may be done by separating the elements of a tissue with needles mounted in handles (Fig. 5), which process is called

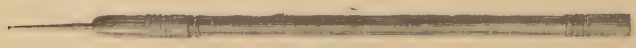

Fig. 5.

teasing, and immersing them in glycerine. To illustrate the process, let us take, for instance, a piece of muscle, as obtained from a fresh piece of beef. A small portion is to be removed with a pair of scissors, cutting

parallel with the fibres of the meat, and is placed in a small dish, or watch glass, together with a drop of glycerine. Then, with a needle in either hand, the fibres are separated from each other, until a number of them have been isolated, when they should be placed upon a clean slide and covered over with a clean, thin glass cover. They are then ready for examination by transmitted light, which means light coming from the mirror and through the sub-stage condenser.

The separation of the histological elements of tissues with needles is a tedious process, and requires considerable patience. When the elements to be isolated in this manner are very small it becomes necessary to carry on the teasing under a magnifying glass, which is so arranged that it can be moved up and down, so as to place it at the proper distance from the object, which latter is placed upon a table or stage having a hole in its centre, through which the light is reflected from a small mirror fastened below. The whole apparatus is known and sold under the name of dissecting microscope (Fig. 6).

Frequently, however, it is desirable to isolate histological elements which are too closely united with each other to be easily separated with needles, under the dissecting microscope. Or, it is desirable to render certain parts of a tissue more transparent, thus revealing other structures, which, by the processes of staining, described further on, cannot be clearly demonstrated. For these purposes chemical reagents are employed, acting either upon thin sections placed under the microscope, when their influence may be observed, or upon thicker pieces

of tissues immersed in the reagent for a longer or shorter time, according to circumstances.

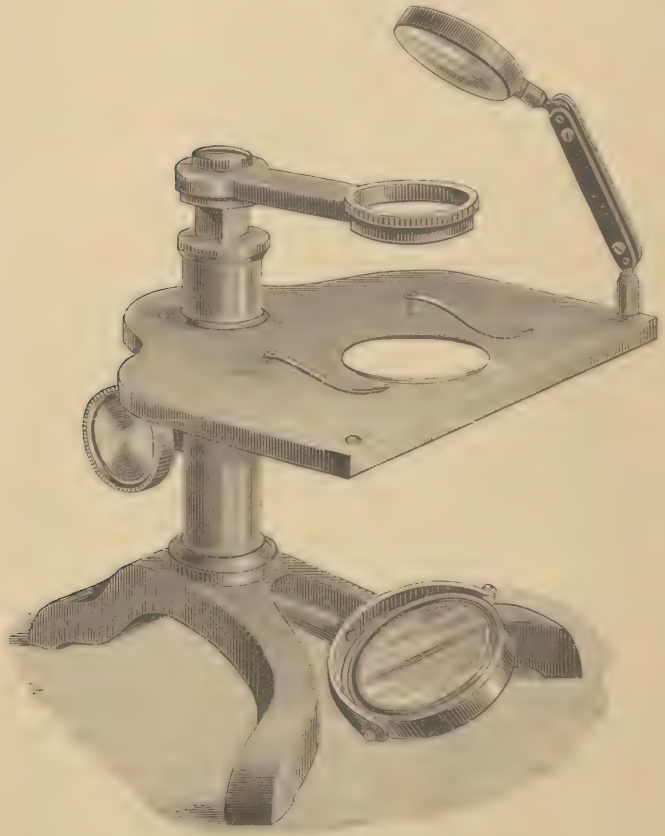

FIG. 6.

These reagents are of two kinds, viz., acids and alkalies, and are employed, as a rule, in very weak solutions.

If the reagent is to act upon a thin section, the latter is placed upon a slide and is covered with a covering glass. The solution of the reagent is then placed in small quantity alongside of the cover, by means of a pipette, and is allowed to run under so as to come in contact with the tissue and act upon it. In order to facilitate the operation, it is well to insert a piece of blotting paper or a cotton thread under the edge of the cover, opposite to the place where the drop of solution has been allowed to run in. In this way a current is established, which in bringing fresh portions of the solution in contact with the tissue, causes it to act more energetically. Larger pieces must be soaked in the solutions for a longer or shorter time, and of varying strength, according to the purpose to be accomplished. Besides isolating several of the elements of the tissues, these reagents are also used with advantage to differentiate some of the normal and pathological formations, by chemically combining with them and forming new compounds of different colors.

ACIDS.

Among the acids most commonly used for the purposes above indicated are—

First. Sulphuric acid (C.P.), which is used in its concentrated state for isolating the cells of cornified epithelium, nails, hair, etc. These tissues must be placed in a few drops of the acid and remain there for a few minutes, when they should be removed and washed in water to which some ammonia has been added, in order to neutralize the acid. It will then be found that the elements of the tissue can readily be separated in glycerine

with needles, and they can then be mounted in either a watery or resinous medium, as will be described below.

A mixture of sulphuric acid and cane sugar, of the strength of one drop of the concentrated acid to one centigram of a five per cent. solution of sugar in water, may be advantageously used to color some organic substances red, such as cellulose, amyloid and albuminous bodies, oleic acid, etc. A weak solution of the acid (one per cent.) renders connective tissue very transparent in from twelve to twenty-four hours, and is thus useful in isolating muscular fibres.

Second. Nitric acid, which is used strong (C.P.) for isolating connective tissue corpuscles, bone corpuscles, dentinal canals, etc., in the same manner that sulphuric acid is used for cornified epithelium; it is, however, necessary that the bone should be cut into thin plates before the acid is allowed to act upon it. A twenty per cent. solution is useful for isolating the organic or smooth muscular fibre cells, which may be accomplished by macerating a piece of the muscular coat of the intestine for several hours in the solution. The action of nitric acid upon the earthy salts of bone in connection with chromic acid, will be mentioned in another place.

Third. Hydrochloric acid. Undiluted, this acid is not used in histological investigations, but is very useful when diluted, for isolating the corneal corpuscles and connective tissue corpuscles. For this purpose a fifty per cent. solution is employed and the tissue is allowed to be acted upon by the acid for several days. The same solution is used in order to isolate the uriniferous tubules of the kidney by dissolving the inter-tubular

connective tissue. To do this successfully, make a section of a piece of fresh kidney with the freezing microtome, and place this for about twelve hours into the acid solution, then wash it in water made alkaline with ammonia and macerate in distilled water for twenty-four hours. The tubules can then be easily separated with needles, under the dissecting microscope, and form a beautiful object. The tubules of other glands may also be isolated in this manner.

Fourth. Chromic acid. This reagent is most commonly used for the purpose of hardening tissues prior to cutting sections from them, and for extracting the earthy salts from bone in connection with other acids, under which heads it will be mentioned. A very weak solution (one-eighth grain to one ounce of water) is, however, used to bring out delicate nerve tissues, such as the touch corpuscles in the skin and conjunctiva, or the prolongations of the ganglionic cells of the central nervous tissues, by prolonged maceration of small portions of these tissues in the solution.

Fifth. Oxalic acid, besides being used in some instances for fixing the carmine after staining, is also employed as a concentrated alcoholic solution to swell up the connective tissue and make it transparent, while at the same time it hardens the albuminous substances; thus it becomes an excellent reagent for demonstrating the rods and cones of the retina and of Cordi's organ. It does not seem to make much difference how long the tissue is allowed to remain in the solution, provided the former has been placed in it as fresh as possible, a few minutes after the death of the animal; for it will be found that

after the lapse of a few hours the desired effect is already produced, while the tissue may remain in the solution for several days without detriment.

Sixth. Acetic acid. A one per cent. solution of glacial acetic acid has the effect of making the connective tissue very transparent in tissues immersed in it, and also of rendering the nuclei of cells apparent. On account of this action it is used to differentiate delicate nerve filaments in the tissues, and by means of this reagent the terminations of nerves in the striped muscular tissue may be demonstrated. To do this, place the pectoral muscle of a medium-sized frog, immediately after the death of the animal, in a solution of two drops of glacial acetic acid in fifty cubic centimeters of distilled water, and allow it to remain for twenty-four hours. The muscle should then be washed in distilled or filtered water and stained in carmine, as described below, and the fibres are then to be separated with needles, under the dissecting microscope, when they may be mounted in glycerine or balsam.

Osmic and picric acids are used more particularly for hardening and staining, and will be mentioned under these heads. Many other acids have from time to time been recommended for a variety of purposes by different workers; but those mentioned above are mostly used in histological investigations, and their action upon the tissues is thoroughly understood.

ALKALIES.

First. Caustic potash. A thirty per cent. solution of caustic potash is very useful in isolating muscular fibres,

nerve elements, ciliated cells, ducts of glands, etc., by dissolving the connective tissue, and for this purpose the solution is allowed to act upon fresh, thin sections of the tissue for about half an hour. The tissue must be examined in this solution, and cannot be permanently mounted, as the action of the reagent continues and cannot be stopped.

Second. Caustic soda. This alkali acts similarly to potash, and is particularly useful in demonstrating the anatomy of hair and nails. It should be used much weaker than the potash, a fifteen per cent. solution being the maximum strength.

Third. Liquor ammonia. The action of ammonia is similar to that of potash and soda, but is most commonly used to neutralize acids.

Various salts are also used with advantage in histological investigations for special purposes, but it will be sufficient to mention here only a few of them.

Chlorinated soda solution, commonly called Labarraque's solution, is chiefly employed to remove coloring matters, such as the chlorophyll in vegetable tissues and black pigment in animal tissues.

Bichromate of potash is used for hardening animal tissues, under which head it will be considered, but may also be used for the same purposes that chromic acid is employed for, especially when a less energetic action is desired.

Cyanide of potassium and hyposulphide of soda, in very weak solutions, are useful in lighting up too deeply stained silver and gold preparations.

The student should provide himself with a set of

glass-stoppered bottles filled with the different acid and alkaline solutions, and should keep a special pipette for each solution, so as to prevent contamination of one solution with another. These bottles should be labeled and kept, together with a few clean test tubes, in a rack, such as chemists use for reagent glasses and test-tubes.

When using the reagents on the stage of the microscope great care should be exercised to prevent injury to either the lenses or the brass work of the stand, and it is advisable to cover the stage with a piece of plate glass, and to use, as much as possible, low powers with long working distances.

The histological elements may thus, by teasing and by the action of reagents, be separated and even isolated, but in normal, as well as in pathological histology, the relations of the elements to each other are often of more importance than the form of the elements themselves, and therefore large portions of tissue must be made thin and transparent, without destroying the relation of parts. This may be accomplished by cutting thin sections of the tissue to be examined. Most tissues are, however, too soft when fresh to be cut into thin sections, and they must be prepared for this process by hardening or freezing.

HARDENING.

There are several reagents which act upon animal tissues in such a manner that they become hard if immersed in them. Those most commonly used among them are, alcohol, Müllers fluid, chromic acid and glycerine, and they all produce the hardening effect by coagulating the albumen and gelatine in the tissues and by abstracting water. It is evident that if this is done

rapidly a very great amount of shrinkage must take place, and if the piece be at all large the hardening agent is prevented from entering into the interior of the tissue, by its own action upon the outside. These reagents should therefore be used with great care, so as to prevent shrinkage and distortion as much as possible. All tissues should be as fresh as they can be obtained, when placed in any of the hardening agents, for if decomposition has set in they cannot be properly hardened, and all subsequent manipulations will be useless, as no good specimen from such material can be obtained.

Alcohol. Hardening with alcohol, which acts by extracting water and coagulating the albumen and gelatine, is to be done as follows: Place a piece of tissue, not larger than a cubic inch, into a wide-mouthed jar containing alcohol of the strength of forty-five per cent., and enough to cover the tissue to the depth of at least an inch. Let the piece remain in this for three days and then place it in a similar jar containing sixty per cent. alcohol. After the lapse of another three days it should be removed to eighty per cent., and after the same length of time, to ninety-five per cent. alcohol, in which it should remain until hard enough to cut. Some tissues require still further hardening in Squibb's absolute alcohol before they have acquired the necessary degree of hardness. This may be tested by taking the piece of tissue between the forefinger and thumb and lightly compressing it. If it feels springy and elastic, like a piece of India-rubber, it should be hardened further by longer immersion in alcohol, but if it feels hard, like a piece of soap, it is ready for cutting.

A great deal of time and alcohol may be saved if the hardening is done in four jars (FIG. 7), holding about one-half pint each,* filled three-fourths full of the different solutions of alcohol, and labeled No. I, forty-five per cent.; No. II, sixty per cent.; No. III, eighty per cent.; No. IV, ninety-five per cent. One or more pieces to be hardened are then labeled, by fastening to them, with a pin, a strip of ordinary writing paper, upon which the necessary remarks should be written with ink, and dropped into jar No. I. After three days they should be transferred to jar No. II, No. III and No. IV, having remained in each one the proper length of time. After several specimens have been hardened, by passing through different jars, it is necessary to degrade each jar to its number below, to throw away the contents of No. I and to fill it with the contents of No. II, and so on in rotation, filling jar No. IV with fresh ninety-five per cent. alcohol. This must be done in order to keep the different solutions up to strength, because water is abstracted from the tissues and makes the alcohol weaker.

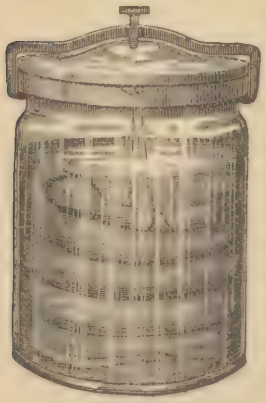

FIG. 7.

It will be found that tissues thus hardened take the different stainings with great brilliancy and are but little shrunk or distorted. For this reason, and because of its

* Whitall, Tatum & Co., of Philadelphia, manufacture a very convenient jar for this purpose, and at a very reasonable price.

quickness and simplicity, this process of hardening is preferred by most microscopists in America. In Europe, on the other hand, alcohol is very expensive and the cheaper methylated alcohol is but little used, on account of its disagreeable odor. European microscopists denounce alcohol in very strong terms, because, they say, it shrinks the tissues too much. And so it does if used too strong at first; by the gradual increase of strength, however, time is given for the alcohol to take the place of the abstracted water, and the process of coagulation goes on so slowly that the decrease in bulk is almost inappreciable.

Almost all kinds of tissues may be hardened in alcohol, with the exception of very young embryonic tissues, myxomata and colloid tumors, which are better preserved in Müller's fluid, or may best be frozen, in order to cut sections from them.

Müller's fluid. Another excellent hardening agent, which acts by the coagulation of albumen and gelatine, but which abstracts only a small percentage of water from the tissues, is called Müller's fluid. It is composed of—

Bichromate of potash,	2½ grams
Sulphate of soda,	1 "
Distilled water,	100 "

and is especially applicable to delicate organs, such as the brain, spinal cord, eyes, embryoes, etc.

The tissue to be hardened should be of small size, not larger than half a cubic inch, and should be immersed in at least ten times its bulk of the fluid, which should be renewed every three or four days. The length of

time required to harden varies with the character of the tissue, and is never less than two weeks, while it may take six weeks or two months to accomplish the purpose. This time may be shortened by finishing the hardening in ninety-five per cent. alcohol after the tissue has remained in the Müller's fluid for two weeks.

In consequence of the slowness of the process of hardening, the tissues are reduced but little in bulk, and their elements are scarcely altered, but this reagent has very little penetrating power, that is, it will not exert its influence upon the deeper portions of the tissues and, therefore, the pieces immersed in it must be small, as otherwise they will be hardened only on the outside, while the centre undergoes decomposition.

Sections cut from tissues hardened in Müller's fluid should be soaked for several days in alcohol before staining, so as to get rid of most of the yellow stain which is imparted to the tissue by the bichromate of potash, as otherwise the color of the staining fluid will not be brilliant and will not properly differentiate the histological details.

Chromic Acid.—Very weak solutions of chromic acid, not stronger than one-half per cent., are frequently used for hardening all kinds of tissues, especially by German microscopists, and they act like Müller's fluid but with more energy. For this reason the time required to harden tissues in such a solution is less than in the case of the Müller's fluid, but the penetrating power is also less, because the very action of the reagent in coagulating the albumen and rendering the gelatine insoluble presents a barrier to its affecting the deeper parts. It will

be seen that on account of this only very small pieces can be satisfactorily hardened in chromic acid solution. This chemical imparts a decidedly green color to the tissues, which even after prolonged soaking in water or alcohol will not entirely disappear, and very materially interferes with the staining of the sections. One great disadvantage of chromic acid solutions is, that mould is very readily developed upon their surface.

Müller's Fluid and Alcohol.—The difficulty of hardening large pieces in any of the above reagents has long been felt, and many other chemicals have, from time to time, been recommended as having the desired penetrating quality, but they have all been found wanting on extended trials. The author, during these experiments, has hit upon the plan of combining several of the reagents, and has finally found that a mixture of equal parts of Müller's fluid and ninety-five per cent. of alcohol produce the desired result. In this fluid whole human brains with their membranes intact, whole kidneys, and other large organs may be hardened throughout and in a comparatively short time. Nerve tissues, especially, are better hardened in this mixture than in either alcohol or Müller's fluid alone.

The manipulations necessary in this process are somewhat different from those described above, and some care is required in carrying out the different steps. The following details, carefully observed, have been found by the author necessary to success.

Immerse a brain or a large piece of another tissue in the mixture of alcohol and Müllers fluid contained in a large wide-mouthed jar, or, better still, an earthen pot holding

about two quarts; cover it with a glass plate and set it in a cool place where the temperature does not rise above 45° F., nor fall below freezing point. In summer it should be placed in an ice chest, and in winter it should be protected from frost. During the first three days the brain should be turned at least twice in the twenty-four hours, in the solution, so as to preserve its form and allow the fluid to act on all portions equally. The fluid itself should be replaced by fresh every day for about two weeks, when it may be changed every three or four days. In the course of five or six weeks the brain will be found to be of sufficient consistence to be handled without danger of injuring it, and it should then be placed into ninety-five per cent. alcohol. This also must be changed every two or three days until it shows but very little discoloration, and the brain will be found hard enough throughout, so that thin sections may be made across both hemispheres. The time thus required to harden a whole brain is about three months, but smaller pieces of tissues may be gotten ready in much shorter time; spinal cord, for instance, will be hard in about two weeks—one week in the mixture and one week in the alcohol alone—provided the fluids are frequently changed. This process seems at first sight rather expensive, on account of the large quantity of alcohol used, but a great deal of it can be used over again, and for this purpose the mixture, when poured off from the specimen, should be filtered, and a little fresh alcohol and some crystals of bichromate of potash should be added, when it may be used again for other specimens to begin with. The discolored alcohol result-

ing from the second part of the process may also be used for either making the mixture or for the sixty per cent. alcohol in the alcohol hardening process.

The tilting of a large jar or pot containing about two quarts of liquid and a brain, which should be handled with care, so as not to injure it, is by no means easy, and it will be found more convenient to draw off the liquid by means of a syphon, which can readily be made of a piece of rubber tubing.

Glycerine. Strong glycerine, as is well known, has a great affinity for water, and thus may be employed as a hardening agent for small pieces of tissue and young embryoes. It should, however, like alcohol, be at first used diluted with water, and the strength gradually increased, as otherwise considerable shrinkage will result. It at the same time makes tissues very transparent, which often is of great advantage and is an excellent preservative, so that the tissues immersed in it do not undergo any decomposition. The same is true of the other reagents already mentioned.

There are a number of other substances used in special cases and for special purposes, such as arsenious acid, picric acid, osmic acid, chloride of iron, chloride of gold, platinum, palladium, and many others; but as this treatise is for the use of the beginner in microscopy we will leave the consideration of these reagents to larger text books, especially as they are not universally applicable to all tissues.

Sometimes a tissue is composed of parts but loosely connected with each other, and yet it is desirable to make a section which shall comprise all these parts and exhibit them in their proper relation to each other, as, for

instance, the inner ear, the larynx, the eye, and others. The ordinary hardening and imbedding is not sufficient to give the necessary resistance to the tissue, and a section, if cut thin enough, will surely break before it is completed, or during the process of staining and mounting. It is, therefore, very desirable to have a means by which this difficulty can be obviated and large sections of such complex tissues may be successfully cut, stained and mounted. This may easily be accomplished in the following manner:—

After the tissue has been thoroughly soaked in alcohol, it is placed in a vessel containing sufficient melted cacao butter to cover it completely. The vessel is then put in the water bath and the temperature is kept for six hours at 130° Fahrenheit. Care should be taken not to let the temperature rise above this point, for a greater heat will injure the tissue and make it unfit for microscopical examination. After the lapse of the time indicated the tissue is placed in the well of the microtome, is imbedded, and allowed to cool together with the imbedding material, which will take some three or four hours. Sections may then be cut with great facility, and they should be stained before an attempt is made to remove the cacao butter from the interstices of the tissue. After staining, they should be placed in absolute alcohol, and when completely dehydrated, are to be placed into a small vessel containing some crude benzole, which will not only readily dissolve the cacao butter but will also make the section transparent, so that it may be at once mounted in balsam. If it is desired to mount the section in a watery medium, it should be removed from

the benzole into alcohol, and from thence into water, after which it is to be treated according to the description given for mounting in a watery medium.

SOFTENING OF BONE.

The student of histology will frequently desire to make sections of tissues containing bone, as in fœtal and some pathological specimens, or ossified cartilage, the removal of which by dissection would defeat the end entirely. It is, therefore, desirable to have some reagents by which the earthy salts may be removed and the bone or ossified cartilage rendered soft, so that it can be cut.

Most of the mineral acids in very weak solution will do this, such as a one per cent. solution of nitric or muriatic acid, but these are not applicable for bone deeply imbedded in soft tissue, although they soften isolated pieces of bone and teeth in a short time.

A saturated solution of picric acid frequently renewed will soften small portions of bone very rapidly, and at the same time harden the soft tissues, but its intense yellow color, and the difficulty of removing stains resulting from even weak solutions, makes it inconvenient to use.

The best formula for softening bone, in the author's opinion, is the following:—

Chromic acid,	1 gram
Nitric acid (C. P.),	2 c.c.
Water,	200 c.c.

Tissues containing bone may be immersed in this liquid and allowed to remain four or five days, when by

piercing the bony parts with a fine needle the student can readily tell by the resistance encountered whether the bone is soft enough or not. If not, a few drops of nitric acid should be added to the liquid, and the specimen allowed to remain therein until the bone is found to be decalcified. The tissue should then be soaked in several changes of pure water for several hours and hardened in alcohol as described.

CHAPTER III.

CUTTING SECTIONS.

There is no doubt that the most satisfactory and most instructive way to study histology, both normal and pathological, is by thin sections, which should be as thin and as large as possible. Various means have been employed to obtain these, and various instruments have from time to time been devised to facilitate the operation of section cutting.

The simplest, but by no means the best plan, is to take a piece of hardened tissue between the thumb and forefinger of the left hand, and to cut thin sections from it with a sharp knife, held in the right hand. If the piece is too small to be held between the fingers it must be imbedded in some substance which, when warm, is fluid, and which becomes hard on cooling, such as wax, paraffine, or soap, or it may be clamped between two pieces of some substance which does not offer any more resistance to the knife than the tissue itself, such as boiled liver, carrot, elder pith, and so forth. This method is employed in most of the laboratories in Europe, but the sections obtained are not as satisfactory as might be wished, and even if they are thin, they are small in extent, and it requires considerable skill to cut even a small section.

With the so-called Valentine's knife, which consists of two small blades that can be separated from each other any desired distance, by means of a small set

screw, sections of fresh tissues may be made by cutting into the piece and floating off under water the section found between the blades. It is, however, nearly impossible to have the blades parallel; they are difficult to keep sharp, and the sections cut with this instrument are usually wedge-shaped. However, Valentine's knife is very serviceable in the post-mortem room when a hasty microscopical examination of an organ is desired.

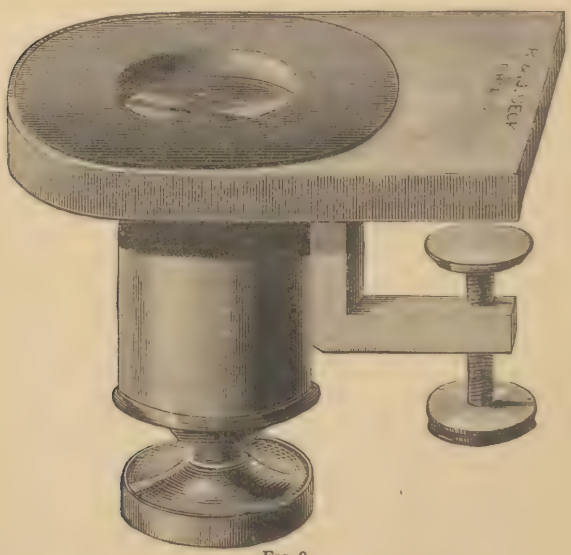

FIG. 8.

The Microtome. The most satisfactory and almost indispensable apparatus for cutting sections is a microtome, and the student who is desirous of saving time, labor and material, will do well to supply himself with one (FIG. 8). Various forms of this instrument are in the

market, the principle, however, being the same in all. It consists of a brass tube, called a well, closed at the bottom by a disk or block, into which is fitted a long screw, having from thirty to forty threads to the inch, and which is turned by a large milled head. This screw as it ascends in the centre of the well pushes before it a loosely fitting false bottom, which is prevented from turning around in the tube by a notch cut into its edge, into which fits a small bar, soldered to the inside of the tube. This tube, with its screw and false bottom, is screwed, at its open end, into a metal plate, which may be fastened to the edge of the table by a clamp, and to its upper surface is cemented a plate of glass having a round hole cut in its centre, a little larger than the bore of the tube and fitting directly over it.

In purchasing a microtome, it should be carefully examined to see that the micrometer screw has no lost motion, or is shaking in its bearings, for upon its *steady* upward pressure depends most of the success of section cutting.

When the microtome is to be used the hardened piece of tissue is laid upon the false bottom of the well, which has been lowered to such a depth that the tissue is below the surface of the glass plate. The well is then filled with an imbedding material, which is fluid when hot and becomes hard when cold, completely covering the piece of tissue. A variety of substances may be employed for imbedding, but the author, after many experiments, has found the following to be more satisfactory than any other, in the majority of cases, because if poured into the well at a temperature of about one hundred

and twenty degrees, it will not shrink away when cooling, either from the tissue or from the wall of the microtome well.

This imbedding material consists of—

Pure paraffine, 2 parts.
Rendered mutton tallow, 1 part.

After cooling, the imbedding material is cut from the surface of the tissue downward and toward the wall of the well, in front and on either side, but is left standing behind. This is done to prevent the knife from cutting through anything but the tissue itself, as otherwise particles of the imbedding material adhering to its surface might tear the section before it is completed. By turning the milled head of the screw in the bottom of the well, the false bottom, and with it the tissue, is raised above the surface of the glass plate to a slight extent, and whatever projects beyond that level is cut off by the knife sweeping over the opening of the microtome.

The Section Knife. An ordinary razor, ground flat on one side, may be used for cutting, but a knife somewhat longer, expressly made for the purpose, is to be preferred. Such a knife should not be wider than one inch; its back should be of considerable thickness; its edge should *be straight*, and it should be well balanced in the handle. One side, the lower one if the knife is held in the right hand, the edge pointing from the person, should be flat and the upper side ground slightly concave or hollow, and its cutting edge should be four times the length of the diameter of the well of the section cutter. There are reasons for all these minutiæ about

the section knife which it is well for the student to know, and we therefore give some of the most important ones.

The edge of the knife should be straight, because in the act of cutting the knife must be tilted somewhat, that is, the back should be raised so that the edge only glides upon the glass plate of the microtome, for if any waves exist on this edge the resulting section will be uneven in thickness. The same will result if the edge is convex, like that of a razor, for then the slightest

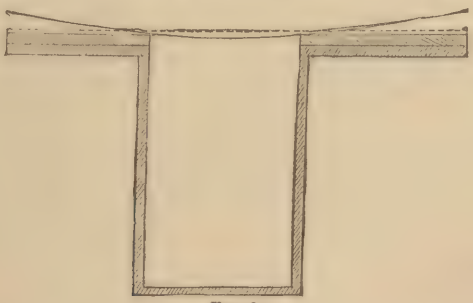

FIG. 9.

change of inclination of the knife during the act of cutting will cause a gouging into the tissue. The accompanying diagram will make this clearer than can be done in mere words.

The *under* side of the knife should be flat, because it is the one nearest the glass plate in cutting from the body. It will be found that it is much more easy to cut away from than toward the body, because the so-called muscular sense, a very important factor in section cutting, is more highly developed in the extensor muscles of the arms than in the flexors.

The knife should be properly balanced in order to insure perfect and continuous contact of the cutting edge with the glass plate, thus avoiding dipping of the edge, which results from unequal pressure at one or the other end of the knife.

Even if the microtome is a good one, and the knife perfect, the student will find some difficulty in cutting sections thin and even, because it requires a very steady hand and a good deal of practice, for if the knife is not carried across the well of the microtome with an even sweep and unchanging inclination, the section will be wavy if at all large. This difficulty has been long experienced, and many contrivances have been constructed to overcome it, with varying success. In order to fully appreciate the difficulty it will be necessary to examine into the process of cutting a little more closely. It will be found that the edge of the knife has a bevel produced by honing and stropping (FIG. 10), which will raise the

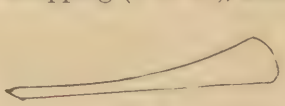

FIG. 10.

cutting edge above the glass plate when the flat side of the knife is laid on the plate, and will cause the knife to cut upward and out of the tissue, thus breaking the section before it is completed; or, if the operator is conscious of this upward tendency, and endeavors to correct it, alternate bands of thick and thin portions will be observed in the section. This difficulty can be obviated by raising the back of the knife slightly, so as to cause it to slide on the bevel. It will be seen, therefore, that

the knife should be carried through the portion of tissue projecting above the level of the glass plate with an even motion and at the same inclination, to insure success.

The Author's Mechanical Knife Carrier. It occurred to the author, some time ago, that if the knife could be rigidly fastened to some apparatus by means of which it could be moved over the well of the microtome in the same manner that the hands move it, sections of any desired size and thinness could easily be cut, even by an

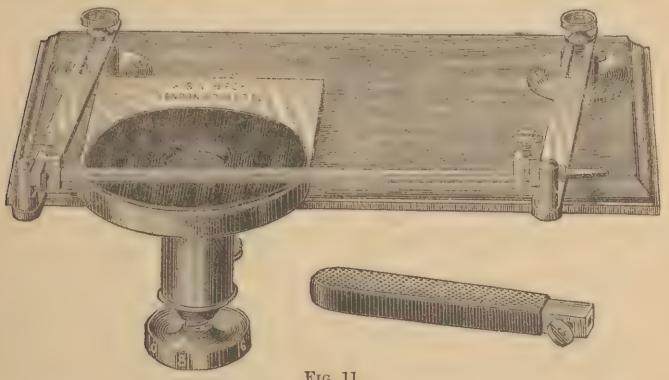

FIG. 11.

unpracticed hand. After some experimenting, an apparatus was constructed which proved to be all that can be desired in an apparatus of this kind (FIG. 11).

It consists of two rigid parallel arms of metal, which at one end revolve on pivots attached to the table to which the microtome is clamped. On the other end of these arms are fastened revolving clamps which hold the knife, the edge of which, when in position, rests upon the glass plate of the microtome. The handle of the

knife is removed, so as to prevent any hindrance to the motion, but can easily be attached by means of a screw, when the knife is to be stropped or honed. When in position and ready for cutting, the edge of the knife is kept in contact with the glass plate by a slight pressure of the fingers upon the upper surface of the blade, and a side motion is given to it by the hands, which causes it to pass through the tissue and cut a thin, even section without any difficulty.

As has already been mentioned, several mechanical microtomes have been constructed by various workers, but to my knowledge they are all deficient in one point, viz., the knife or cutting instrument in these is carried through the tissue like a chisel, or, in other words, the cutting edge is *pressed* through the tissue. But the knife, in order to cut well and evenly, must be carried through the substance to be cut—especially if it is soft—in a slanting direction, so that each point of the edge describes a curve which is equal to a part of a circle. By referring to Fig. 11, it will be seen that in the author's apparatus this is exactly what takes place when the knife is moved, the radius of the curve being the length of the arms from the centre of the pivots to the centre of the clamps holding the blade.

As has been several times remarked in these pages, it is of the greatest importance, in normal as well as in pathological histology, to examine large sections, so as to obtain a clear picture of the relation of parts to each other. Especially is this true when the histology of organs which are very composite in their nature is studied, and where it becomes necessary to identify large

agglomerations of elements, as is the case, for instance, in the brain, the spinal cord, the larynx, the kidney, etc., or in composite pathological new formations. How whole organs can be prepared for the cutting has already been mentioned, and it remains to describe the process of cutting large, thin sections of such organs, as, for instance, across the whole human brain.

It is self evident that sections of the size indicated cannot be cut in the ordinary manner, as they would break before they are completed, even when cut comparatively thick. Sections thin enough for even the higher powers may be cut under a liquid, preferably alcohol, so that they are floated off as soon as they are cut, and are caught upon a piece of stiff writing paper, upon which they remain throughout all subsequent operations of staining and mounting.

This necessitates a special apparatus, which is constructed as follows: Take a microtome which has the required size for the organ to be cut (for a human brain the microtome well should be about eight inches in diameter), and fasten it, water tight, in the centre of the bottom of a trough lined with zinc, which should be six times the diameter of the well in length and three times in width, and its side should be about one inch high (inside measurement). The knife, which should be three times the length of the diameter of the well of the section cutter, is to be mounted on arms or levers of wood shaped like a **Z**, in the same manner as is done in the author's mechanical knife carrier. These levers are swung on pivots, which are fastened on the table some distance beyond the trough, so as to obtain sufficient

swing for the knife. Those pivots should be of the proper height, so that the knife has the necessary inclination toward the glass plate of the microtome, as was described above.

When everything is in readiness the brain is to be imbedded in the well of the microtome, the trough is filled with alcohol so that it stands about a quarter of an inch above the glass plate, and after the imbedding material has been cut away in front and on either side of the specimen, the knife is carried through the tissue with one sweep. The resulting thin section should at once be floated on a piece of stiff writing paper and removed to a vessel containing alcohol. The ordinary tin plates used for baking pies will be found well adapted to this purpose. After a sufficient number of sections have been cut, the levers carrying the knife may be unscrewed from the pivots and removed from the trough, and the latter may be covered with a large plate of glass, to prevent the evaporation of the alcohol, so that sections may be made at some future time.

It is a good plan to number the pieces of paper upon which the sections are floated and manipulated, because then a record can be kept of the approximate position of the section in the organ, and this number can be inscribed upon the label of the finished preparation.

In cutting sections, whether by hand or with the mechanical microtome, the edge of the knife should always be in contact with the glass plate, and should be advanced with a steady motion, for the slightest hesitancy or pause in the motion will alter the inclination of the knife in hand cutting, and the resulting section will be uneven.

A prerequisite to good section cutting is that the knife should be as sharp as it can be made, and the student should learn to hone the knife properly, so as to be independent of the cutler. After a few sections of any tissue have been cut, the knife should be stropped, so as to keep its edge in proper condition, and it should be well cleaned before putting away, after the requisite number of sections have been cut, so as to preserve its keen edge.

The Freezing Microtome.—There are some tissues which will not bear the action of hardening agents, such as myxomata and colloid tumors, and others, so that if sections are to be cut from them, they must be hardened in some other way. This may be done by freezing them, either in a mixture of ice and salt, or by means of the ether or rigoline spray, in a freezing microtome. This, if the ice and salt mixture is to be used, consists of an ordinary microtome, the tube of which is surrounded by a box or tank which is filled with the freezing mixture. A few drops of a thick solution of gum arabic are placed on the false bottom of the well, and the piece of tissue is placed thereon. In a short time it will be found frozen hard enough to cut, when very thin sections may be made from it. The knife should be kept cold and dry, otherwise the sections will adhere to its surface, and are then difficult to remove without tearing them.

A more simple and convenient apparatus is the spray freezing microtome (Fig. 12), in which the piece of tissue is placed upon a drum, which can be raised by the screw in the microtome, so that the specimen projects beyond the surface of a second plate of glass which is

mounted upon pillars and fastened to the metal plate of the microtome. This drum has a hole in its side, and through it the atomizing tube is introduced, thus causing the spray of the volatile liquid to produce the cold within the drum and cause the piece to freeze solid and fast to the lid. All condensed liquid is collected in the

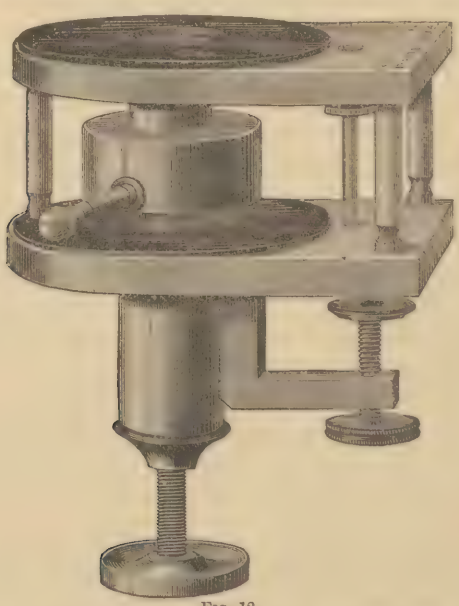

FIG. 12.

bottom of the drum and may be withdrawn by means of a stop-cock.

There are numerous modifications of the different apparatus described, each one claiming advantages over the others; but as the purpose of this little volume is to guide the beginner, and not to bring everything new in

microscopical technology before the public, this consideration may safely be left out. Those described the author has thoroughly tested and found to answer all purposes.

Every tissue requires a little different management in cutting, which can only be learned by experience; it is, therefore, advisable for the student to practice on one tissue until he is able to make satisfactory sections of it before going to another one. Thus, if he takes, for instance, the stomach of a medium-sized frog after it has been hardened in alcohol, as has been described, and cuts sections from it until he is able to obtain extremely thin and even sections all the way across the organ (about one-third of an inch in diameter) he will have made more progress in a shorter time than if he had taken different tissues and cut them up.

The question is often asked how thin should the sections be cut? The answer to this must be that the sections should be as thin as the elements will hold together, and that this thinness varies with every tissue. Sections of injected tissues, that is in which the capillaries have been filled with a colored liquid, so as to make them more apparent, should be made thicker, in order that the ramifications and loops of blood vessels may be clearly seen, which in too thin a section are cut away, leaving only those vessels which happen to run parallel with the plane of the section. Actual measurement of the thickness of the sections is altogether unnecessary, although it may be made by means of the fine adjustment of the microscope, in the following manner: Focus the lens upon the lowest plane of the

section, which must be perfectly flat upon the glass slip, and then turn the fine adjustment until the uppermost layer is in focus; now count the number of threads of the fine adjustment screw to the inch, note the number of degrees that the milled head has been rotated, and the distance traversed by the lens will be found by a simple calculation; this distance being equal to the thickness of the section. It is impossible to measure with accuracy the thickness of the section by the micrometer screw of the microtome, although it seems easy enough, because there are so many factors which enter into the production of the section, that gross errors of measurement are very readily introduced.

Bone Sections. Sections of hard substances, such as dried bone, teeth, and even of rocks, may be prepared thin enough to be examined by transmitted light and with high powers. Such sections are made in the following manner: Cut with a fine saw, such as is used for scroll sawing, as thin and as large a piece of the macerated and dried bone or tooth as is possible; rub one surface of it upon a fine sandstone until an even surface is obtained. This is then to be polished, by rubbing upon a fine Arkansas hone, using plenty of water during the operation, and is to be finished by rubbing upon a piece of soft leather, which has applied upon its surface some jeweler's rouge, until a fine polish is obtained and all the scratches made by the first coarser stone have disappeared.

A small piece of evaporated Canada balsam is then heated upon a glass slide, over a spirit lamp, and the piece of bone or tooth is pressed into the softened

balsam, with the polished side downward; the slide is then again heated until the balsam boils, and the specimen is pressed down with considerable force, so that it will lie flat upon the glass. After the balsam has cooled and become hard, the surplus is scraped away, and the piece of bone, which has tightly adhered to the glass, is rubbed upon the sandstone, and ground as thin as possible. Great care must be exercised to hold the glass slide parallel with the surface of the stone, as otherwise the section will be ground unevenly and will be wedge-shaped when finished. When thin enough, the section is polished upon the hone, and finished upon the leather with rouge. The grinding and polishing should be done with circular strokes and not with a to and fro motion of the hand, in order to avoid deep grooves being made into the section by coarser particles of the stone.

The section, when thus polished on both sides, is to be thoroughly washed with a stream of water from a syringe or under the tap, and is then to be examined under the microscope, to see whether it is satisfactory as regards thinness and polish. When found to be too thick or uneven, it must again be put on the sandstone and the fault remedied by further grinding. If found perfect a drop of balsam is to be placed upon it, after all the water has evaporated, and it is to be mounted in the manner described below.

This process is not as difficult and tedious as might appear from the description, especially when the student has access to a lathe. In that case the work of grinding and polishing is done very quickly and easily, and

several pieces may be ground at once, by cementing them side by side upon a piece of glass, and holding them against the revolving grindstone. They must then, of course, be transferred, when finished, upon separate slides, which can readily be done by the application of a little heat.

CHAPTER IV.

STAINING OF TISSUES.

By staining the tissues or thin sections of tissues, is meant the tinging of certain parts, for the purpose of differentiating the different elements from each other by their color, or by the varying intensity of color. This may be accomplished in a variety of ways and by a variety of colors, and depends upon the peculiarity of certain tissue elements to absorb and retain the coloring materials more readily than others. Thus the nuclei of cells will take up and retain certain vegetable colors whereby they are made distinctly visible under the microscope. This faculty is lost entirely by the nuclei after decomposition has set in, or partially after the tissue has been acted upon by certain reagents, as, for instance, chromic acid, and for this reason the tissues should be placed into the hardening agent as fresh as possible. Strange as it may seem, tissues which are perfectly fresh or still living will not stain, and must first be killed, so to speak, by some reagent, such as glycerine or alcohol, before their nuclei will take and retain the coloring matter. Other elements beside the nucleated structure may be differentiated or made distinct by coloring matters or chemical reagents, so that often a combination of colors may be produced in a specimen, each of which maps out, so to speak, the elements which have an affinity for it, and this is termed double staining, because usually only two different color-

ing materials are used, which may, however, produce in the section a much larger number of colors or shades of color.

Carmine. Among all the different coloring matters, carmine is best retained by the nuclei, and for this reason, and also because it does not fade, it is most extensively used for staining. Many workers in this department of microscopy have devised a large number of methods of employing this color for staining, and to give a description of them all would only mislead the student, who does not know which one to use for his purposes. We shall, therefore, give only one, which has proved to be certain and satisfactory in most cases, and which is very simple in its manipulations. This method was originally devised by Prof. Tiersh, but has been modified and improved by Dr. J. J. Woodward, of Washington, and is as follows:—

Take of—

Best carmine, No. 40,	15 grains.
Borax,	1 drachm.
Water,	5½ ounces.
Alcohol (ninety-five per cent.),	11 ounces.

Mix and filter. The liquid which runs through the filter paper is but slightly colored and should be thrown away as useless. The crystals, however, which will be found remaining in the filter should be carefully removed and dissolved in eight ounces of distilled water. In order to save time and trouble, the filter, together with the crystals on it, may, as soon as the filtration is completed, be thoroughly mixed with the eight ounces of water, in a mortar. The solution is then to be

filtered and evaporated in a water bath, to four ounces, when it is ready for use.

The sections which, after cutting, have been kept in alcohol, are then taken out and placed into a small dish filled with this lilac-colored liquid, for a short time, from thirty seconds to one minute being sufficient; but they may be left in it for several minutes without fear. They are then placed into what is termed a fixing solution, composed of—

Hydrochloric acid (C.P.),	1 part.
Alcohol,*	4 parts.

and allowed to remain therein until their color has changed from a lilac to a rose color. The action of this fixing solution is to remove the excess of color and to bleach all other parts, except the nuclei, which retain the carmine staining in spite of the action of the acid, so that they alone are brilliantly colored red. This is, in many cases, of great advantage, as a much clearer picture results than if the coloring is also seen in the non-nucleated structures, and the nuclei are only marked by deeper staining. If, however, it is desired to stain the cell contents and intercellular tissue also, this can be accomplished by employing a weak solution of hydrochloric acid in alcohol (ten drops to the ounce), or by using a saturated solution of oxalic acid in sixty per cent. alcohol, as a fixing solution. The sections are then removed from the acid solution and are washed in several changes of alcohol, which should be of varying strength.

* Whenever the term alcohol is used in this volume without definitely giving its strength, pure ninety-five per cent. alcohol is meant.

Thus, the first washing should be sixty-five per cent., the second eighty per cent., and the last ninety-five per cent., in order to prevent shrinkage of the tissue by the action of strong alcohol upon it after it has been saturated with water absorbed from the staining solution. If tissues are stained which do not require hardening, such as membranes, and are to be mounted in watery media, the alcohol is dispensed with and the fixing solution is made with water while the washing is also done in water.

Tissues, whether they be in sections or as thin membranes, will be found on examination to be evenly and brilliantly stained.

Hæmatoxylon. Another very valuable staining material is hæmatoxylon, which is prepared by mixing a strong watery solution of the extract of logwood with a concentrated solution of alum, or by treating logwood with alum-water, as made and sold by Bullock & Crenshaw, a large chemical house of Philadelphia.

Tissues to be stained are immersed for some ten or fifteen minutes in a strong watery solution of alum, to which are then added a few drops of the hæmotoxylon solution, sufficient to give a deep purple color In the course of five or ten minutes more the sections are stained uniformly of a violet color, which on examination under the microscope will be seen is chiefly retained by the nuclei, very similar to carmine. The specimens are then to be washed in water for a few seconds and placed in a small dish or watch glass, filled with alcohol, wherein they should remain at least twenty-four hours before mounting.

If the section is placed directly from the alcohol into the hæmatoxylon solution, a precipitation of the coloring matter by the alcohol will occur, and the section will either not be stained at all, or will be filled with granules of the hæmotoxylon. Also, if the sections are not, previous to staining, immersed in the alum solution, which acts as a mordant, the staining will be uneven, and, therefore, unsuccessful.

One great drawback to this method of staining is that the color is not permanent, and will fade, especially if the specimen be mounted in glycerine. However, as long as it lasts, it gives very satisfactory results, and is, in many cases, even preferable to carmine staining. A good plan is for the student to stain two sections of the same tissue, one with carmine and the other with hæmatoxylon, and compare the results.

Double Staining. After a section has been stained with carmine, as described above, the cell contents and fibrous portions may be stained with a blue color, obtained from indigo, which will tinge some parts a decided blue, others green, and still others by a combination of the red of carmine with the blue, of a violet color, this producing a beautiful effect. This is the more valuable as different tissues invariably are tinged by the same shade of color, so that thereby they may be recognized and fine distinctions may be made by the aid of color, which are impossible to make in single stainings. The necessary manipulations are as follows: Take a neutral solution, in water, of either indigotate of potash, commonly called indigo carmine, or of the sulph. indigotate of soda, and add a few drops of it to sixty per

cent. alcohol, until the liquid assumes a dark sky-blue color. Place in this the section, which has previously been stained with carmine, and thoroughly washed, for from four to twelve hours, according to the rapidity with which the tissue takes up the blue color, as this varies greatly in different tissues. When the section has assumed a decidedly blue color, if examined in clear alcohol, it is sufficiently stained, but the exact time when it should be removed from the indigo solution can only be determined by experience. On examination under the microscope, after having been made transparent, the section should show its nuclei bright red, while the connective tissue, muscular fibres, cell walls, if such be present in the cells, blood disks, etc., should be tinged with a variety of shades of blue. A quicker, but not quite as certain a method of obtaining the same results is to immerse the carmine-stained section in a saturated watery solution of oxalic acid, to which the concentrated neutral indigo solution has been added, in the proportion of fifteen drops of the latter to one ounce of the former. The sections are first stained with carmine, using the watery acid solution for fixing the color, and are thoroughly washed in water; they are then immersed for a few minutes in this indigo solution, when they will be evenly stained with the blue. If, on examination, the blue is too dark, the surplus may be removed by soaking the sections for some time in a saturated solution of oxalic acid. After this the specimen should be carefully washed in several changes of water, so as to remove the oxalic acid, for if any trace of it remains in the tissue, both the blue and the red color will fade in a short time. This

latter method of double staining is not as certain, because the blue is apt to combine with the red in the nuclei and give them a violet hue, which materially detracts from the brilliancy of the staining and from the contrast of colors.*

There are a number of other methods of staining with two or more colors, such as with picric acid and carmine, hæmatoxylon and carmine, aniline colors, and so forth, but they are by no means certain, nor are they as satisfactory as the process described above, and we therefore will not give a description of them.

It is evident that isolated cells, bacteria, portions of teased tissues and so forth, cannot be stained in the manner just described, and that if color is to be imparted to them, it is to be done on the slide on which they are mounted. For this purpose the aniline dyes are preferable to any other coloring material, and should be used in very weak watery solutions, the fuchsine and iosine solutions being perhaps the best to use, but other aniline colors may be employed.

To stain isolated cells or bacteria, without displacing them from under the thin glass with which they have been covered, place a drop of the staining solution on the slide, close to the edge of the cover, and allow it to run under by capillary attraction, then place a piece of blotting paper close to the opposite edge of the cover, to establish a current. This done, put the slide under the microscope and observe the action of the coloring mate-

* This process was devised by the author, and was published in the *American Quarterly Microscopical Journal*, Vol. I, No. 3, April, 1879.

rial upon the cells; when they have been sufficiently stained, remove the drop of coloring solution and substitute for it a drop of distilled water, which by passing under the cover washes away all superfluous coloring material and leaves the cells or teased tissue stained. The mounting material, which should be a watery medium, is then in the same manner introduced under the cover, and the slide is finished as described below.

Nitrate of Silver. Some portions of tissues have an affinity for salts of metals in solution, and precipitate the base in its metallic form, or as an oxide, by the aid of light. Thus the cell cement connecting epithelial and endothelial cells may be stained black by the oxidation of silver, in the following manner: Place a membrane or thin section of a fresh tissue, made with the freezing microtome, in a half per cent. solution of pure nitrate of silver, and allow it to remain until it appears milky. It is then to be washed in distilled water and exposed to the action of diffused daylight, in a shallow dish of porcelain, filled with water, to which a few drops of acetic acid have been added, so that it becomes slightly acid. The action of light will reduce the silver salt to its oxide, and the tissue becomes brown, when it should be washed in a weak solution of table salt, in order to convert the unreduced nitrate of silver into the chloride, and should then be soaked for a few moments in a weak solution of hyposulphite of soda (two per cent.), with a view of removing the chloride and preventing further darkening of the specimen by the action of light. In a successfully silver-stained specimen the epithelial or endothelial cells will be mapped out with great dis-

tinctness, but frequently the silver will be deposited in the cell itself, instead of in the cell cement, and then the tissue presents a mass of cells containing small black granules of silver, without having their outlines definitely marked by black lines. A tissue successfully stained with silver should be afterwards stained with either carmine or hæmatoxylon, in order to bring out the nuclei.

The cornea of a frog or rabbit may be very successfully stained by painting it with a strong solution of nitrate of silver (forty grains to one ounce of water), while the animal is alive and under the influence of chloroform, and the white skin of the belly of a frog thus treated makes beautiful preparations, showing the epithelial cells and the stomata between them.

Chloride of Gold. For differentiating delicate nerve filaments, and for bringing the corneal corpuscles into view, a one per cent. solution of chloride of gold is an admirable reagent. The tissue, *as soon as possible*, should be immersed in the gold solution until it assumes a straw color; it should then be washed in distilled water and exposed to diffuse daylight for several hours, in some acidulated water, until it has assumed a reddish-violet color throughout, when it is to be washed again in water, and may then be stained with indigo solution, before mounting.

Osmic Acid. In order to stain the ganglionic cells of the spinal cord, as well as their processes, so that they can be isolated, a thin slice of fresh spinal cord is to be soaked for several days in a one per cent. solution of osmic acid, until it assumes a brownish color; it should

then be washed in water and placed for twenty-four hours in dilute glycerine, when it may be teased out with needles, and the ganglionic cells, which are stained of a deep brown if the staining has been successful, may be isolated. The red-blood cells of animals may also be stained with osmic acid, so that they may be easily seen when mounted in balsam. In order to do this, prepare a blood slide, as described in the section on illumination, and place it, with the blood cells downward, over a shallow vessel containing a two per cent. solution of osmic acid, thus exposing the blood corpuscles to the influence of the vapor of the acid. After a few minutes the slide may be prepared for mounting (as will be described below), and after some time the blood cells will assume a distinctly brown color, thus making them clearly visible. Osmic acid will stain fat cells of a deep brown, almost black, and thus becomes a valuable reagent when a differential diagnosis is to be made between fat and other substances resembling it under the microscope, which are not thus stained.

CHAPTER V.

INJECTING THE VASCULAR SYSTEM.

In order to clearly demonstrate the vascularity of a tissue and to show the ramifications of the capillaries, the blood vessels must be injected with some colored fluid which will remain in the vessels after the tissue has been hardened and sections have been cut from it. This fluid should hold the coloring matter in *suspension* in a finely subdivided state, and not in solution, so as to prevent the escape of the coloring matter through the stomata of the vessels into the neighboring tissues.

There are two injecting fluids which have given the most satisfaction in the hands of the author, the one of a red color and the other blue. This red fluid is made as follows :—

Take of—

Best carmine, No. 40,	2 drachms.
Distilled water,	3 ounces.
Strong liquor ammonia,	20 drops.

Dissolve this and filter through cotton, covering the funnel with a piece of glass plate, to prevent the evaporation of the ammonia. The filtration is a somewhat tedious process but is absolutely necessary ; the solution, however, will keep, and may therefore be kept in stock.

Then take of—

Coxe's gelatine,	2 drachms.
Distilled water,	2 ounces.

Soak the gelatine in the water until it becomes soft, and

then dissolve it in a water bath and strain through fine flannel while hot. Heat the gelatine solution again and add the carmine solution, and bring the temperature up to about two hundred degrees, Fahrenheit. Dilute acetic acid must then be added, drop by drop, under constant stirring of the mixture, until the ammonia is just neutralized, which is indicated by a sudden change of color in the solution, from a lilac to scarlet. If the acid is added too quickly or too concentrated, the precipitation of the carmine is too sudden, and the granules become so large that they cannot pass the smaller capillaries, thus preventing the success of the subsequent injection; and if acid is added after the solution has become neutral the gelatine will not congeal in the vessels, and the fluid will run out when the sections are made. It will be seen, therefore, that some experience is necessary in preparing this fluid, and the student should not feel discouraged if he fails in his first attempts, for repeated trials will give him the necessary skill, and he will be rewarded by the beauty of his preparations. This fluid must be used hot.

Beale's Blue Injecting Fluid. Another injecting fluid, which is to be used cold, is Beale's finest blue injecting fluid;* it is prepared as follows:—

Take of—

Chloride of iron (E.PH.), 10 drops.
Glycerine, 1 ounce. Mix.

Then dissolve three grains of ferrocyanide of potassium in a little water and add to it half an ounce of glycerine. Mix the two solutions and add, under con-

* "How to Work with the Microscope," third edition, page 200.

stant shaking, drop by drop, half an ounce of water, acidulated with three drops of muriatic acid (C.P.) which precipitates the coloring matter in exceedingly fine granules. The only drawback to the use of this injecting fluid is that it fades very soon, unless mounted in an acid medium, and even then it is not staple. But as the same holds good of other fluids prepared by Prussian blue, and as this gives the seemingly only available blue coloring matter, and as they have other disadvantages, this fluid is the best to use. It might be here stated that it is commercially manufactured by Bullock & Crenshaw, of Philadelphia, and may be obtained from them.

THE METHOD OF INJECTING.

The instruments necessary for the injection of the capillary system are a dissecting case in good order and a syringe of metal holding about two ounces, and of such a length that if the piston is drawn out to its full extent it can be pushed down by the thumb of the same hand that holds the barrel. The plunger should fit the bore of the barrel air tight, but should move easily and without jerks. The syringe should be provided with a removable stop-cock, to which several nozzles of different sizes can be fitted. These nozzles should have a little bulb at the point, and should be provided at the other extremity with a hook-like projection to which the thread used in tying the nozzle into the vessel is fastened.

The best animal to inject, for the beginner, is a young cat, because its blood vessels seem to be tougher and

withstand the necessary pressure better than those of most other small animals.

Possessing the dissecting case and syringe, having procured the cat, and having prepared about twenty ounces of the red carmine injecting fluid, the student may proceed to the operation of injecting as follows: Place the cat into a box, together with a sponge saturated with chloroform, and then close the lid. Very soon the animal will be quiet, when it should be placed upon the table and an incision made into the skin in a line with the sternum and reaching from the clavicle to the end of the sternum. This incision is best made with a sharp pair of scissors, as a knife is apt to slip in the thick fur of the animal. The skin and underlying muscles are then to be dissected from the sternum and intercostal cartilages, and the thoracic cavity is opened by cutting through the intercostal cartilages on either side and bending the sternum upward, just as it is done in making a post-mortem examination of a human subject. Care should be taken not to sever the mammary arteries, and everything should be done as quickly as possible.

The thoracic viscera having thus been exposed, the pericardium is picked up with a pair of forceps and slit open, and the apex of the heart, thus disengaged, is cut off with a pair of scissors, so that both ventricles are opened. The animal must now be thoroughly drained of blood, by lifting it alternately by the ears and by the hind legs, until no more blood runs from the heart. The largest of the nozzles of the syringe that can be introduced is then pushed through the left ventricle of the heart into the aorta, and the latter *only* is tied around

the nozzle behind the bulbed end, with a piece of stout suture silk, the free ends of which are fastened around the hook-like projection of the nozzle and drawn tight, so as to prevent its slipping out. It is best to fill the nozzle with injecting fluid before introducing it into the aorta, so as to prevent the introduction of air into the vascular system, which might prevent the successful injection, or cause extravasation by bursting some of the vessels. The syringe is then filled with the injecting fluid, which has been heated to a temperature of about 130° Fahr., the stop-cock attached to the syringe is introduced into the nozzle, opened and held firmly in position with the left hand while the plunger is gently, but with a steady motion, depressed by the thumb of the right hand. As soon as the fluid reaches the capillary system, a resistance to its progress will be felt by the operator, and this must be overcome by a slight increase of pressure upon the plunger of the syringe. But as soon as it is overcome the pressure should again be diminished to the degree used at first. When the syringe is emptied the stop-cock is to be closed, the syringe disengaged from it and re-filled. Its permanent nozzle is then reintroduced into the stop-cock and the injecting proceeded with until the injecting fluid, which has driven the blood remaining in the vessels before it, runs out pure from the right ventricle. The stop-cock is then again to be closed, and all the vessels coming from the heart are to be tied with another piece of suture silk, around the nozzle, and after opening the stop-cock, a little more injecting fluid is forced into the vessels. This is done in order to over-distend the vessels, so that

when the gelatine, on cooling, contracts, it will fill the vessels completely.

If, during the injection, the fluid is seen to ooze from any vessels which have been cut, they must be tied, or if they be small the leakage can be stopped by picking up their extremities with a pair of forceps and twisting them.

The injection completed, which, with a medium-sized cat, should not take longer than five minutes, the stop-cock is to be closed, the syringe removed from it, and the animal placed in ice water for at least six hours before the nozzle and stop-cock are removed, or it is dissected; for the gelatine must be given time to become solid, as otherwise the injecting fluid will be forced out of the smaller capillaries by the contraction of rigor mortis.

If any of the injecting fluid is left over it may be set in a cool place with a small piece of camphor in it, and will thus keep for several days. Or it may be poured on a large glass plate, also placed in a cool place (in summer on ice), and when it has become solid the mass may be cut into narrow strips, detached from the glass plate and hung up to dry, when it will keep indefinitely. One ounce, by weight, of this dried material should be soaked in ten ounces of water, then dissolved in a water bath and strained through a salt bag which has previously been boiled, before using it again for injecting. As a general rule it will be found that the lungs of an animal treated as described above are either not injected at all or but little of the fluid has found its way into them, and they should be injected separately by tying the nozzle into the pulmonary artery, before the

vascular system of the rest of the body is injected. An animal which has been dead for some time, and in which the rigor mortis has just passed away, or a human fœtus, or even a separate organ of an animal or man, such as a leg or arm, may also be injected with this fluid. In order to do so the animal or organ should be placed for several hours into warm water, and the same should be injected into the vessels, in order to wash out any clots of blood before proceeding as described above for the recently killed animal. In the case of a separate organ, the nozzle should be introduced into the largest artery, and all other vessels should be tied, except the largest vein, as the injecting fluid makes its appearance at their cut extremities. The largest vein should also be tied after the fluid has flown from it for some little time, and all the vessels should then be over-distended by a little more pressure upon the piston of the syringe, for the reason given above.

The cold-flowing blue injecting fluid may be used in the same manner as the red, with the exception that it is not necessary to warm dead animals or detached organs before injecting ; or it may be used, for the latter class of objects, with a so-called pressure apparatus, the simplest and most convenient form of which consists of a funnel attached to a flexible rubber tube. In using such an apparatus, one of the nozzles belonging to the syringe is to be introduced into the artery, after having been filled with the injecting fluid; the stop-cock is then attached to one end of the rubber tube and the funnel to the other ; the funnel is filled with the injecting fluid and raised to such a height that the hydrostatic pressure

will be sufficient to force the liquid through the vessels, when the stop-cock is to be introduced into the nozzle and when opened the injecting proceeds without further labor on the part of the operator.

This method of injecting, although very convenient and efficient for the blue fluid, cannot be employed when the red is to be used, because the gelatine would become cold and congeal in the funnel; and neither the red nor the blue can be used with the pressure apparatus in recently killed animals, because of the momentary increase of pressure necessary to overcome the resistance felt when the fluid enters the capillary network.

Double injections, in which the arteries are injected with the red fluid and the veins with the blue, can be made in detached organs only by injecting the red fluid first through the artery until the fluid has reached the capillaries, which is known by the increased pressure necessary to overcome the resistance. The artery is then tied and the nozzle introduced into the vein through which the blue fluid is injected. If the experiment has been successful, sections made from the organ will show the arteries red and the veins blue.

Another injecting fluid must be mentioned, which is used in order to demonstrate the outline of the endothelial cells lining the blood vessels in the frog. It is made as follows:—

Nitrate of silver, 3 grains.
Distilled water, 2 ounces.

Dissolve and mix with a hot solution of—

Coxe's gelatine, 1 drachm.
Distilled water, 1 ounce.

The frog is to be put under the influence of chloroform by injecting some of it under the skin of the back, and the heart is to be quickly exposed and cut in the same manner as was described when treating of injecting a warm-blooded animal. After all blood has been drained out of the body, the fluid is injected while warm, and all thin membranes, such as the mesentery, bladder, and so forth, are removed and exposed, in acidulated water, to the action of light; in fact, they are treated as if they had been stained with silver.

After the injection of any of these fluids all the instruments and vessels used should be carefully washed in hot water, so as to have everything in readiness for the next time injecting is to be done, for nothing is so annoying as to have everything in readiness and find that the piston of the syringe will not move up or down because the nozzle is stopped up with dried injecting fluid, or to find the same state of imperviousness in the stop-cock.

In order to obtain satisfactory preparations of injected membranes, such as the mucous membrane of the urinary and gall bladder, the mucous membrane of the intestine and stomach, the choroid of the eye, etc., it is necessary to remove the epithelial cells, which, in a great measure, interfere with a good view of the blood vessels. To do this, place the membranes, or the tissues to which they are attached, into Müeller's fluid, diluted one-half with water, as soon as the injecting fluid has set, if gelatine has been used as a body, and let them soak for two or three days. Then gently wash them in clean water, and soak them for twenty-four hours in dilute glycerine.

The membranes may then be separated from the underlying structures, and the epithelial cells can be removed by brushing the surface of the membrane in glycerine with a sable brush, such as is used by artists for water-color drawings.

If the tissues have been hardened in alcohol, it is very difficult to separate the membranes, and almost impossible to remove the epithelial cells. This is especially the case in the membranes of the eye and the pigment cells of the choroid.

CHAPTER VI.

MOUNTING AND FINISHING OF SPECIMENS.

After the section or thin membrane has been stained, it must be made transparent before it can be examined by transmitted light and can be mounted or preserved permanently, but as this process is part of mounting we will first describe the necessary tools employed.

The student should provide himself with a pair of fine-pointed nickle-plated forceps; with which to lift the sections from one solution to another; also with several needles, already mentioned in the section on teasing. Further, some slips of white plate glass with ground edges, in size three inches long by one inch wide; some cover glasses, preferably circles, three-fourths and seven-eighths of an inch in diameter, of No. 2 thickness, and a turn-table.

Cleaning Slips and Covers. The glass slips and covers must be chemically clean before they can be used for mounting. When new, both slides and covers are more or less greasy, from being handled by the workmen, cutting, grinding and packing them, and no amount of rubbing, either with a dry, soft rag or moistened with alcohol, will remove this thin film of oil; besides, the thin covers are too frail to be handled roughly. The slips should, therefore, be placed for a few hours in a solution of—

Bichromate of potash,	2 ounces
Sulphuric acid,	3 fl. ounces
Water,	25 fl. ounces.

in such a manner that the brown liquid covers them completely. They are then to be rinsed under the tap, and stood on edge, on several thicknesses of blotting paper. When dry they are chemically clean, and require no further manipulation except to brush off any dust which may have fallen on them, with a soft camel's hair brush. Care should be taken not to touch their surfaces with the fingers, nor to breathe upon them, as this would soil them again.

New covers are treated in the same manner, placing an ounce or so of them in a wide-mouthed vial, covering them with the cleaning solution, and shaking them up occasionally, so as to separate the covers from each other, and allow the solution to act upon their surfaces. After several hours, the solution is to be poured off and the covers washed in the bottle, with water, pouring it on and off, until it remains colorless, after continued shaking with the covers in the bottle. The water is then to be carefully drained off, and alcohol is poured on the covers, wherein they remain until wanted for use. The bottle should be tightly corked, to prevent the evaporation of the alcohol.

When it is desirable to clean off specimens which have been mounted, either in balsam or in a watery medium, so as to save both the cover and the slide, the specimen is to be slightly warmed over a spirit lamp, and the cover is then to be pushed, with a pair of forceps or needle, over the edge of the slip into a vessel containing alcohol and hydrochloric acid in equal proportions. The surface of the slip is then scraped clean of the remaining balsam or cement, and dropped into the

bichromate of potash solution, where it should remain several days, after which it may be washed under a tap, and set up to dry on blotting paper, as described for cleaning new slips.

The covers must remain, also, for several days in the acid alcohol, and must then be carefully washed under the tap, to get rid of the disintegrated film of balsam adhering to them; and after this transferred to the cleaning solution and treated like new ones.

Turn-table. A turn-table, which is used for centering the object on the slide, for making cells upon it, in which to mount in watery media, and for building up cement rings around the edge of the cover, consists of a circular disk of brass, mounted with its central axis in bearings, so that it will revolve rapidly and easily. These bearings are fastened to a stand, made either of wood or of cast iron, which, when placed upon the work table, gives a support for the right hand holding the knife or brush. Upon the brass plate are spring clips for holding the slide in position, or if the turn-table is a self-centering one, two diagonally opposite corners of the slip are held in clamps, which, by a mechanism, move toward and away from the centre of the plate in like ratio. The self-centering turn-table (FIG. 13), although a little more expensive than the other kind, will be found to be much more satisfactory, as a great deal of time is lost in centering the slide by hand, which is also somewhat difficult for persons not accustomed to it. There are several patterns of these turn-tables in the market, the best of which are those whose centering mechanism consists of three toothed wheels, fitting into

each other and revolving on the under side of the brass plate, or those in which the clamps are approached to or receded from the centre by a lever. The stand should be provided with a little bolt at the right side, which by

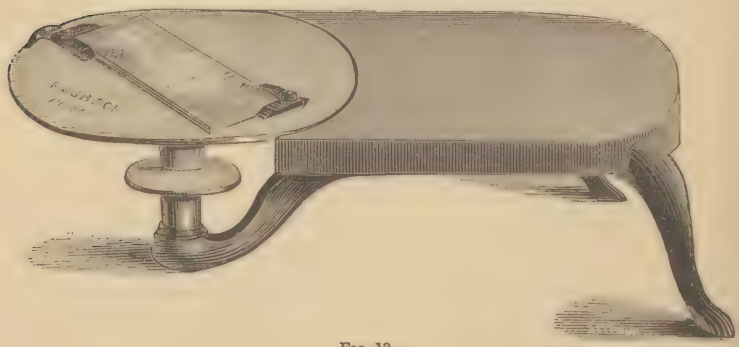

Fig. 13.

being pushed forward stops the revolutions of the disk, so as to prevent its turning while the slide is being placed in position.

MOUNTING.

For mounting, both resinous and aqueous solutions may be used, which each possess advantages over the other, and for this reason a controversy has been going on for some time, between eminent microscopists, in regard to the advantages of glycerine, on the one hand, representing the aqueous mounting media, and balsam, on the other, representing the resinous class. The truth is, that both should be used, as occasion requires. Glycerine, or its equivalents, should be used when it is desired to bring out delicate striæ, lines, hair-like projections, such as cilia on the epithelium of the respiratory

tract, processes of the ganglionic nerve cells, and so forth, and for delicate vegetable preparations. Balsam should be used when clearness and transparency of the object, and brilliancy as well as durability of the staining is desired.

In order to clearly understand this, the student will do well to mount two preparations of the same tissue, the one in balsam, or other resinous medium, and the other in glycerine or its equivalent, and then compare the results. He will find that the one medium is better suited for a particular preparation than the other.

Balsam. Among all resinous substances Canada balsam is the best for mounting purposes, provided it has been properly prepared. To do this, take a clear sample of balsam and evaporate it in a water bath, to dryness, that is, until, when hot, all odor of turpentine has disappeared, and, when cold, it is hard and brittle, like resin. This will take several days; and great care should be exercised in keeping the water bath full of water, for as soon as the temperature in the balsam is raised above 212° F. it turns brown, and is then unfit for use.

When thus evaporated the balsam is again heated in the water bath and enough of Squibb's absolute alcohol is added to dissolve it and make the solution of the consistence of thin syrup. It is now allowed to cool and poured into a spirit lamp, the wick having been removed, in which it is kept for use, the glass cap of the lamp protecting it from dust and preventing the evaporation of the alcohol. If, after using for some time, the solution becomes too thick, it should be warmed by

placing the spirit lamp in warm water and adding to it some warm absolute alcohol. If the alcohol used in dissolving the balsam or in diluting the solution is not strong enough, a white precipitate will form, which may be redissolved by the application of heat, but will reappear when exposed to the air, in a thin layer on the slide, and the solution thus becomes useless for mounting.

Having cleaned his slides and covers, and having his balsam solution prepared, the student may now proceed to mount the objects in the following way: Place one of the stained sections which have been kept in alcohol in a small shallow dish containing some absolute alcohol, and allow it to remain there for some minutes, so as to remove all traces of water which may remain in it from the staining fluid and which have not been removed by the washing in the weaker alcohols. Then float it on the surface of some oil of cloves, also contained in a shallow glass or porcelain dish, until it has become transparent, when it should be removed from the oil, spread out on a glass slide and covered with a thin cover glass which has been taken from the bottle filled with alcohol and wiped dry with a soft rag. The specimen is now ready to be examined under the microscope, in order to see whether it will pay to permanently mount it in balsam. If found good the cover glass is carefully removed and all superfluous oil remaining on the section and on the slide is taken up with the edge of a piece of blotting paper, the object covered with a drop of the balsam solution, a fresh, dry cover is placed upon it, taking care to exclude any air bubbles, and pressure is made upon the cover, to press out all superfluous balsam. In order

to prevent the formation of air bubbles in the specimen the cover should be held by the forceps, near the edge, the opposite edge should be carefully placed into the balsam and the cover gradually lowered over the section until it lies flat upon it. If, after pressing the cover down, it is found that the balsam does not extend to the edge of the cover all round, a small drop of balsam should be placed near the edge, at the point where the balsam under the cover joins the empty space, when it will run in by capillary attraction. The slide is then laid aside to allow the balsam to dry spontaneously, which will take place in from four to six weeks, or it may be placed in a drying oven, the temperature of which is not raised above 130° F., when it will be ready for finishing in a much shorter time. An excellent apparatus of this kind is sold by dealers in microscopical appliances, and is shown in FIG. 14. It consists of a box of copper containing movable trays, surrounded by another larger box, also of copper, so that a space remains between the two boxes, which, when the oven is used, is filled with water through an opening at the top. A thermometer is inserted through this opening, and a lamp is placed under the outer box, which raises the temperature of the water up to any desired degree, and thereby warms the air in the inner box. A current of air is established through the inner box by ventilators, both at the top and bottom. Specimens which have been double stained with indigo should not be exposed to either heat or sunlight, as they will fade under these circumstances.

The fact that the oil of cloves or other volatile oils which may be used in its stead shrink many of the more

delicate tissues, and the difficulty attending the removal of large thin sections from one solution to the other, as well as the danger of tearing while they are spread upon the slide, has led the author to discard the oil of cloves

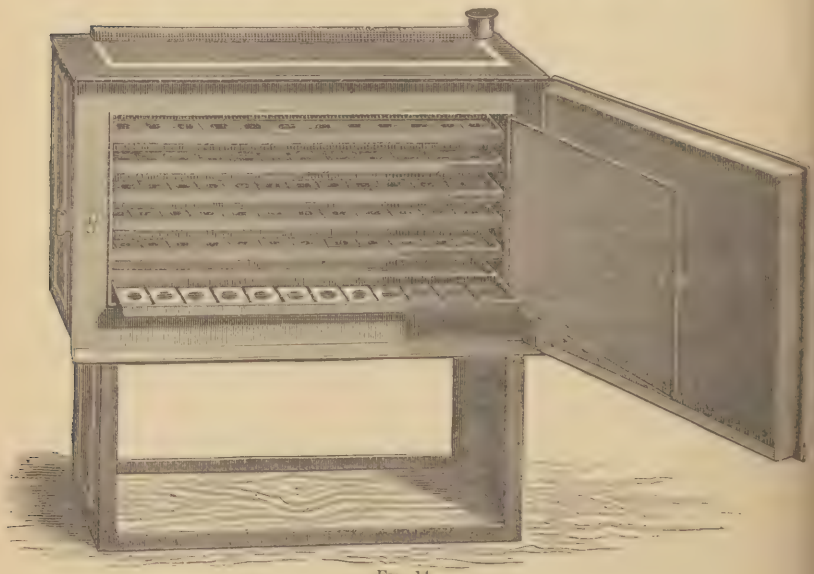

Fig. 14.

as a clearing agent, and to adopt a plan of mounting in balsam, which avoids all these dangers and which has the advantage that the slides may be finished immediately.

THE AUTHOR'S METHOD OF MOUNTING IN BALSAM.

After one of a number of sections which have been stained together has been examined in oil of cloves, and has been found to be good, the others may be inferred to be also good and worth mounting. One of

them is placed in absolute alcohol, and after it has remained therein for some time, is floated upon a cover glass, which need not be wiped dry after taking it from the bottle of alcohol in which the covers are kept, held in a pair of forceps whose ends have been bent so as to stand at right angles to the shafts, and to close on top of each other. (See Fig. 15.) The cover with the section

Fig. 15.

on it is then lifted out of the alcohol, when the specimen will be found to be evenly spread out, needing but little unfolding at the edges, which sometimes fold over; the lower surface is to be wiped dry and a drop of the alcoholic solution of balsam is placed on the section, which, on the cover, is set aside in a place free from dust, to clear up and allow the balsam to get dry. After fifteen or twenty minutes another drop of balsam should be placed upon it, in order to prevent drying of the tissue. After twelve hours the balsam has dried sufficiently on the cover, so that the specimen can be mounted, in the following manner: Take the cover up with a pair of forceps and place a drop of crude benzole* on the balsam and quickly place the cover, with the balsam down, on a clean slide, as near the centre as possible, and taking care to avoid air bubbles. Then warm the slide over a spirit lamp, place on the turn-table and quickly centre the

* The refined benzole or benzine, which is frequently sold for benzole, is too volatile for our purposes.

cover so that its edge does not seem to shake when the slide is rapidly revolved. Next run a ring of cement around the edge, as will be described presently, and then press gently upon the cover, to cause the section to lie flat, and to press out the surplus of balsam, which, with a little management of the pressure, will run into the ring of cement. Another ring of cement may then be applied, when the slide is ready to be labeled and put away.

The cement for balsam mounting which is most satisfactory was devised by Mr. T. W. Starr, of Philadelphia, and is prepared as follows:—

Clear Canada balsam,	370 grains.
Deodorized benzine,	140 grains.
Spirits turpentine,	120 grains.
Gum dammar,	185 grains.

Mix the balsam and benzine well together in a bottle, then add the turpentine and shake until mixed; finally, add the gum dammar, in selected pieces, and shake frequently till dissolved. If necessary, the solution should be filtered through absorbent cotton previously moistened with turpentine. A portion of this is to be placed in a small, glass-capped vial, to the cap of which is attached a small *sable* brush, which will come to a point, the ordinary camel's hair brush not being suitable for ringing, as it spreads too much. If the solution is too thick to flow readily it should be diluted with spirits of turpentine until the proper consistency is obtained. This fluid is also an excellent mounting medium when the object has previously been cleared in oil of cloves or turpentine. For ringing, this cement may be colored

by adding to it a few drops of alcoholic solution of aniline, of any shade desired, or it may be mixed with white zinc, when the resulting ring will appear as if made of porcelain.

The specimen to be ringed is placed upon the turn-table, and if any balsam has soiled the slide or the cover it must be removed by scraping with a sharp knife and afterward wiping with a soft linen rag wet with benzole. As a matter of course, the balsam should be hard, so that the cover will not be displaced by the scraping and wiping. If the cover should not be in the centre, and a self-centering turn-table is used, the slide is to be warmed until the balsam becomes soft, when the cover may be centered on the turn-table. Having thus prepared the slide, the brush in the cement bottle is removed and the surplus scraped off, so that it is almost dry; with the left hand the turn-table is spun around rapidly and the point of the brush applied to the edge of the cover for a moment only, holding the brush slanting in the right hand, and that hand resting upon the stand of the table. The brush is then moistened a little more with the cement and again applied to the edge of the cover, without, however, allowing the hair of the brush to touch the glass; the small drop at the point of the brush only should be in contact with the glass and be carried around by the rapid spinning of the turn-table. The slide is then set aside so as to allow the ring to become thick by evaporation of the benzine and turpentine, when the applications of cement may be repeated until the desired thickness is obtained.

If colored or white zinc cement is to be used it should

not be applied until after the first rings of clear cement have become hard, as otherwise the colored cement will run in under the cover and be disseminated among the mounting medium. If white zinc cement has been used, it may be still further improved by running one or two fine lines of asphaltum varnish around it, but not before the cement has thoroughly hardened.

The making of a neat ring around the edge of the cover is an art which can only be acquired by practice and experience, and therefore a few hints in regard to the causes of failure will greatly help the beginner.

If the ring, when finished, shows irregularities both at its inner and outer edge, the cement used is too thick and should be diluted with turpentine. If the ring is too broad—wider than about one-thirty-second of an inch, unless intentionally widened—the brush has been pressed down too hard upon the glass, which causes it to spread, or too much of the cement has been applied at once. This is especially the cause when irregularities or bulging in the edges of the ring are noticed.

If the ring is filled with minute air bubbles, the brush has been kept too long in contact with the glass in making the first ring, or its point has been brought in contact with the first ring in making the second application, when only the minute drop should have touched the glass; or, finally, the solution may be too thick.

MOUNTING IN GLYCERINE.

When a preparation is to be mounted in glycerine, it should, after having been stained, be placed in dilute glycerine for twenty-four hours, and then for the same length of time in strong glycerine (Bower's), in order to

make it transparent. The section is then carefully spread upon the slide; a clean cover, which has been wiped dry, is placed upon it and pressed down, to remove the excess of glycerine from under the cover, and a small spring-clip is applied, so as to hold the cover in position during the subsequent manipulation of washing. The excess of glycerine must now be removed as carefully as possible, by washing it off with a stream of water, either from a syringe or from a tap, taking care not to displace the cover in doing so. The slide is then stood on edge to dry, the spring-clip still holding the cover, and when all the water has evaporated it is ready for ringing. In order to do this the spring-clip must be removed, the slide placed upon the turn-table, the cover centered and a ring of some water-proof cement applied, in the manner described above.

The best cements for this purpose are, first, the so-called Bell's cement, which may be obtained from any dealer in microscopical supplies, and the composition of which is a secret with the makers; and second, the author's gelatine cement, which is prepared as follows:—

Take of—

Coxe's gelatine,	2 drachms.
Gum ammoniac,	10 grains.
Acetic acid, No. 8,	1 ounce.

Dissolve the gum ammoniac in the acetic acid and filter through absorbent cotton; then warm the acid and gum solution by placing the vessel containing it in a water bath and add the gelatine, stirring until it is dissolved, when the resulting solution should be filtered or strained through muslin. After a ring of this cement has been

made around the edge of the cover, and has become set, it should be painted over with a solution of bichromate of potash in water (ten grains to the ounce) and exposed to either sunlight or ordinary daylight. The action of the light is to make the chromate of gelatine which has been formed insoluble and thus perfectly waterproof. After this gelatine cement ring has become hard, it should be covered with a ring of white zinc cement, when it will be found that none of the glycerine will leak out, even after the specimen has been kept for years.

If thicker pieces than thin sections, such as pieces of the mucous membrane of the intestine or bladder of animals, are to be mounted in glycerine or other watery medium, a cell must be employed. This consists of a ring made of either glass, rubber, metal or cement, and which is high enough to prevent the cover-glass from pressing upon the specimen when mounted. In order to do so, the ring, if it be of glass, rubber or metal, is first cemented upon the slide with marine glue or the gelatine cement, and is accurately centered with the turn-table. If the cell is to be made of cement a ring of the required diameter (which must be a little smaller than the diameter of the cover-glass) is spun upon a slide, in the same manner as was described for applying the ring to the edge of the cover in finishing slides, and is built up to the required height by repeated applications of the brush. It should then be set aside to dry and harden. Any of the cements may be used for this purpose, provided they will stick well to the glass.

Just before mounting the top of the ring forming the

cell must be moistened with gelatine cement; the specimen, which has been made transparent by soaking in glycerine, is then placed in the centre of the ring, enough glycerine is added to fill the cell and the cover is applied. If an air bubble is left under the cover the latter must be lifted up and more glycerine must be added; if, on the other hand, too much liquid has been used, the surplus must be washed off, as described above. A ring of gelatine or Bell's cement is next spun around the edge of the cover, in order to seal up the cell, and it is then finished with white zinc cement.

An excellent substitute for glycerine is Farrant's solution, which combines all the advantages of glycerine and some of those of balsam, inasmuch as it has nearly the same index of refraction as glycerine, and becomes hard like balsam, doing away with the necessity of a waterproof cement. The formula generally given in the text-books for this solution is not correct, and the author has found that the following formula is more satisfactory:—

Picked gum arabic,	4 drachms
Camphor water	4 fl. drachms
Glycerine,	2 fl. drachms.

Dissolve and strain through muslin.

Specimens to be mounted in this medium must first be made transparent by soaking in strong glycerine, and may then be mounted as though the solution were a resinous mounting medium. Great care should be taken to exclude air bubbles, as they cannot afterward be gotten rid of. This medium is especially adapted for delicate animal membranes and soft vegetable tissues.

Some specimens, especially vegetable, such as seeds, pollen grains, sections of wood, etc., may often with advantage, be mounted dry, *i. e.*, without much previous preparation and without any mounting medium, but they must then be examined as opaque objects, and must be viewed by reflected light.

If an object is to be thus mounted, a disk is painted with asphaltum varnish in the centre of the slide, which is spun around upon the turn-table while applying the brush with varnish. A disk of dead black paper may be pasted upon the slide, instead of the disk of varnish, to serve as a dark background. A cell ring is then applied around the edge of the disk, and the object is fastened in the centre of the cell by means of mucilage or glue. This done, the cover is placed upon the ring and cemented down as described above.

CHAPTER VII.

THE PREPARATION OF VEGETABLE TISSUES AND INSECTS FOR MICROSCOPICAL EXAMINATION.

This volume, small as it is, would not be complete without a short description of the methods employed by the most successful workers, for the preparation of vegetable and insect tissues, especially so, as they are not only pleasing to the eye, but also very instructive.

Like preparations of animal tissues, vegetable specimens must be stained in order to bring out the different structures, and should for this purpose be soaked for some time in alcohol; in fact, it is best to place the plants or parts of plants, as they are collected, into a wide-mouthed bottle, filled with alcohol. In this way the chlorophyll is extracted from thin tissues, without injuring the cell contents. For the sake of clearness, we will suppose that the student has collected a variety of specimens of plant life, such as fern leaves, flower stems, ovaries of flowers, and so forth, and that they have been in alcohol for several days. He must now sort them for further treatment; the stems, ovaries, roots, etc., should be placed in fresh alcohol prior to cutting sections from them; the delicate leaves of flowers are sufficiently decolorized to be ready for staining, while the thicker leaves of ferns must be further bleached in Labarraque's solution of chlorinated soda. This reagent removes not only the coloring matter, but also the cell contents, which is a great drawback, but without its use a satisfactory preparation of

thicker leaves cannot be made, and we must, therefore, be satisfied with the results thus obtained. Great care should be exercised in bleaching, and the specimen must be left in the solution only long enough to remove the color, as otherwise the tissue itself will be destroyed. After the leaf has been bleached, all traces of the chlorinated soda must be removed by washing in several changes of water, when it will be ready for staining. Very thick leaves, such as those of the ivy, rubber plant, olive, etc., cannot be made transparent, even with the use of Labarraque's solution, and the epidermal layers must be separated from the thick pulp between them. This may be readily done by cutting, with a pair of scissors, a piece from the edge of the leaf, about one-fourth of an inch deep, and one-half of an inch wide. This piece is then placed into a test-tube containing a few drops of a solution of equal parts of nitric acid (C. P.) and water, and the tube is held over a spirit flame until the solution comes to a boil. It must then be quickly poured into cold water, when it will be found that the epidermal layers can readily be separated from the pulp, and can be opened like the leaves of a book by the aid of two pairs of delicate forceps.

Stems, roots, ovaries, etc., must be imbedded in a microtome and thin sections must be cut from them in the same manner as from animal tissues.

STAINING.

Having thus prepared the specimens they are ready for staining, in the following manner: Immerse the bleached and thoroughly washed leaves or the sections in a saturated solution of alum in water, for from twelve

to twenty-four hours; then place them in a weak solution of the hæmatoxylon staining fluid, which has been mentioned in the chapter on staining of animal tissues, and allow them to remain therein for from eighteen to twenty-four hours, until they exhibit to the naked eye a uniform purple color. It is impossible to give any definite directions in regard to the strength of the solution to be used, or the length of time the specimens are to remain in it, because no two leaves or sections take the color with the same rapidity or facility, and experience alone, gained by repeated trials, can be depended upon. A preparation successfully stained in this way should, under the microscope, show the nuclei, as well as the cell-walls, deeply and uniformly stained of a purplish-blue color.

Double Staining. My friend, Dr. J. G. Hunt, of Philadelphia, devised, several years ago, a method of staining vegetable preparations with two or more colors, which gave such uniformly excellent results that it has now been universally adopted as far superior to single staining. This method, for the detailed description of which I am indebted to my friend, Mr. E. B. Crane, of New York, is as follows: After the specimens have been thoroughly washed in water they are immersed in a very dilute watery solution of methyl aniline green (brand J.J.J.J.*).

Aniline dyes are very powerful, and if allowed to act too rapidly will stain objects unevenly, besides staining

* Any aniline dye soluble in water will probably act in the same way as the brand recommended, but aniline colors insoluble in water should under no circumstances be used for this purpose.

parts of the tissue which it is not intended that this color should touch. If allowed to act slowly (say for at least twenty-four hours in a weak solution), the color will pass into the secondary deposits and formative material, leaving the nuclei and cell-walls untouched. Aniline seems to have a great affinity for new structure; hence, in ferns, for instance, the spores absorb the color with great rapidity. After the specimens have taken up sufficient aniline, that is, when they appear of a uniform color, they must be immersed in a strong solution of alum in water and remain there for twenty-four hours or more. This is done to remove any of the aniline color which may have settled in the cell-walls and to prepare the tissue for the subsequent staining with hæmatoxylon, in the manner described above.

The specimens, after having been stained, may be mounted either in balsam, or in glycerine, or Farrant's solution, according to the plan described for animal tissues.

Insects. Insects, whether they be large or small, hard or soft, may be mounted as microscopical preparations, in the following manner: After the insect has been caught, place it, with as little handling as possible, under a small vessel with a few drops of ether, in order to kill it. When dead, wet it with alcohol and place it in liquor potassa of the strength of one ounce (Troy) fused caustic potassa to one pint of water. Let it soak in this solution until the skin or external part is soft and the internal substance in such a condition that, by slight pressure, the insect can be evacuated by the natural or, if necessary, by an artificial opening. This is best done

under water, and the best vessel in which to do it is a white plate. After this is accomplished, the insect is to be cleaned under water, by holding it down with a camel's hair brush in one hand, and by brushing every part of it, on both sides, with the other hand; then float it on a glass slide and arrange each part in a natural position. This glass slide is then to be covered with another one of the same size, and the two are to be gently pressed together, using only sufficient force to make the insect as thin as possible without crushing or destroying it. The two glass slides with the insect between them should next be tied together with fine brass wire, and placed for from twenty-four to thirty-six hours in clean water. This will give the object a position which is not easily changed, and it is therefore important that the insect be placed in the position it is to have when mounted, before placing it in the water. After having remained in water the proper length of time the insect is to be removed from between the two slides, under water, and is again to be brushed with a soft camel's hair brush, after which it is allowed to float in the water for several hours.

The next step is to again confine the object between two slides, and to place it into a vessel containing absolute alcohol, in which it should remain about twenty-four hours. When the insect has been thoroughly dehydrated by the alcohol, it is released from between the two slides and floated on a cover-glass, held in the mounting forceps, and is mounted in the alcoholic solution of balsam, in the manner already described.

Insects may also be mounted in the dammar medium,

the formula of which was given under the head of ringing; but they must then, after having been dehydrated in strong alcohol, be cleared in oil of cloves or turpentine before mounting.

Considerable patience is necessary in carrying out the different steps of the process, and the utmost cleanliness is required in order to produce satisfactory preparations; but the results obtained, if the process is faithfully carried out in all its details, amply repay for the time, patience and labor spent in producing the specimens.

I am greatly indebted to Mr. Charles Zentmayer and to Mr. T. W. Starr for many of the points given above, in the description of the process for mounting insects.

CHAPTER VIII.

PHOTO-MICROGRAPHY.

All workers in microscopy, doubtless, appreciate the necessity of correctly recording, not only in writing, but also by means of pictures or drawings, many of the appearances seen in the field of the microscope. We can do this by drawing an outline of the objects observed, by the aid of the *camera lucida;* but not only does this require some practice, but also a considerable amount of time, and even then the resulting picture will not be a correct representation of the field of the microscope, because it will always be tinged more or less by the imagination of the draughtsman, and will be more or less diagramatical in consequence.

With photography, on the other hand, an exact reproduction of the image thrown upon the screen can be obtained, and in much less time than it takes to make even a comparatively simple drawing with the *camera lucida.* It is the object of this chapter to give an idea of the means employed to obtain a photographic picture of a microscopic object—means which are in the hands of every microscopist, and which do not require a great outlay of money.

A room with a southern exposure, which can be darkened; a mirror, movable in all directions, outside of the window; an achromatic combination of lenses of from eight to ten inches focal length; a microscope which can be tilted so as to be horizontal, and a stand

to hold the screen and sensitive plate, are all the apparatus absolutely necessary beside the chemicals used in ordinary photography.

These different pieces are disposed of as follows (Fig. 16): The mirror (*a*), which should be eight or ten inches

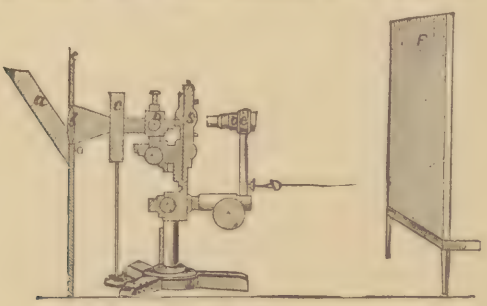

Fig. 16.

long by about four inches wide, is attached to a board which fits into an opening in the dark shutter of the southern window, and is to be moved by rods, from the inside. Instead of this mirror, or in conjunction with it, a heliostat is of great advantage to throw the light of the sun constantly in one direction, for, if once adjusted, it need not be disturbed, and thus a great deal of time is saved. Until recently such an instrument was too costly for the use of students, but of late Mr. Kuebel, of Washington, D. C., has put a heliostat in the market which works very satisfactorily and which is sufficiently low in price to be within the reach of many who desire to work in photo-micrography. The board in the shutter has in its centre a circular opening containing an achromatic combination of lenses (*b*), such as the back combination of a one-fourth portrait photographic lens.

The microscope is secured on the window sill, in a horizontal position, so that the axis of the tube is in a line with the axis of the achromatic combination, and at such a distance from it that the burning focus is about half an inch from the back combination of the sub-stage condenser (*d*). The eye piece is then removed from the microscope, and the tube lined with black velvet, to prevent internal reflection, as far as possible, and the whole apparatus is covered with dark cloth, to prevent stray rays of light entering the darkened room. This done, the sun's rays are reflected from the mirror outside the window, through the achromatic combination, which acts as a concentrator and throws a powerful light through the condenser, through the object on the stage (*s*), and thus a brightly illuminated image is formed by the objective (*o*) on the screen, which latter, when the negative is to be taken, is replaced by the sensitive plate.

This image, when thus formed, must be focused with the greatest care and accuracy, in order to obtain a sharp negative; and as the screen must be at some distance from the microscope in order to obtain the necessary magnification of the object, it is necessary to have some contrivance for turning the fine adjustment at a distance. For this purpose it will be found that a small pulley, placed alongside of the microscope, having an endless band running over it and the milled head of the fine adjustment, answers the purpose very well, when the axis of the pulley is connected by means of a universal joint to a fishing-rod, which by its sections can be made longer or shorter, thus bringing its end close to the screen.

The tube of the microscope, even when all internal reflection has been obliterated, still remains a drawback, inasmuch as it reduces the size of the image, or rather the disk of light, the more, the longer it is. There are, however, some stands made in which the tube can be entirely removed, such as the old Ross stand, and they are therefore very desirable for photo-micrographic purposes.

Any good objective of wide angular aperture and good definition can be employed for photography, provided monochromatic light is used in making the negative. When such is the case the visual and chemical foci fall in the same plane and a special correction of the objective for photography becomes unnecessary.

Such a light is obtained by passing the rays of the sun through a cell containing a strong solution of ammonio-sulphate of copper (*c*) before they enter the substage condenser. I have found some difficulty in making the cell containing this solution, as the copper salt will dissolve almost any cement, and if exposed to the action of the air, very rapidly becomes decomposed, and the solution is thereby rendered useless for the purpose. I have used with satisfaction a cell made of a brass ring, lined on its inner side with lead or tin, having a thread cut on its outside, to which flanged rings are secured. Upon the edges of the inner ring a ring of rubber packing is applied, and upon it a disk of plate glass is laid, which is tightly pressed upon the rubber by the flanged ring. Thus a cell is obtained very similar to the round, flat spirit levels, and which will hold the ammonia sulphate of copper solution for months without change.

In filling the cell care should be taken to leave room for a small air bubble, for if the cell is completely filled the heat of the sun's rays will expand the solution sufficiently to cause leakage.

This solution, besides giving monochromatic light, at the same time filters out almost all the heat rays from the light, so much so that an immersion lens may be used for any length of time without the drop of water evaporating.

At the present time, when dry plate photography has been developed to such an extent that it has superseded, in a great measure, the wet process, it has been thought that it would be the most simple, economical and satisfactory for photo-micrography; but after repeated trials by myself, as well as many others working in the same direction, it has been found that it is not only more expensive, but also takes more time, in the long run. The reason of this is that it is impossible to judge, with any degree of certainty, as to the actinic power of the light forming the image on the screen by merely looking at it, and that a trial plate only will give an idea of the length of exposure necessary for a given day, time of day, objective, and subject, to be photographed. It is true we can expose a dry plate for trial, but then we must develop it immediately, and the time of developing a dry plate is about three times that of developing a wet one, and a dry plate is also about three times as costly as a wet one. Therefore the old wet collodion process is the best.

The collodion to be used should be an old one, and contain some free iodine. I have found that a mixture

of "Anthony's red labeled" and "McCollin's delicate half-tone" collodions—both commercial articles—some five or six months old, gives very satisfactory results. The nitrate bath should contain forty grains of nitrate of silver to the ounce of water, and should be slightly acidulated with nitric acid. The developer should be a weak one: twelve to fifteen grains of the double salt ammonio-sulphate of iron to the ounce of water, containing a few drops of a solution of gelatine and acetic acid as a restrainer.

After the negative has been fixed in the usual way, with hyposulphite of soda or cyanide of potassium, it is almost always necessary to intensify it, which is easily done by flowing the plate while wet with a watery solution of iodine until the film becomes white; then it is to be washed under the tap and flowed with a solution of sulphide of ammonium, which imparts to the negative a dark brown color, and thus strengthens its printing quality.

The object to be photographed should be as thin as possible, because the lens will depict only one plane of it, and it should present as much contrast and differentiation of its elements as possible; this is especially the case in animal tissues, and when high powers are used, the focus should be taken with the greatest care *for one particular point to be brought out;* a general focus, not particularly sharp in any one point, will not give a satisfactory negative.

The screen upon which the image is focused should be of plate glass, having an extremely fine ground surface on one side—the side next to the object. Such a

surface can easily be prepared by flowing the glass plate with a good negative varnish, and when this is set but not yet dry, lightly breathing on it, when an extremely fine and even frosting of the surface will show itself, sufficient to arrest and reflect the rays of light forming the image.

In photo-micrography, as well as in ordinary microscopy, proper illumination of the object is of the greatest importance, and frequently a poor objective will show a better definition in the hands of a skilled manipulator than the best objective can when the light is not properly managed. In this one point lies the difficulty of photo-micrography, and it is the stumbling block over which so many fall who undertake to photograph microscopic objects.

As a general rule the best light is obtained when the back lens of the sub-stage condenser is about half an inch beyond the burning focus of the larger condenser in the shutter, that is about eight and a half inches from this condenser, and when the light is *absolutely central.* But this distance cannot be strictly adhered to, inasmuch as different objectives require different illumination. In practice, I find that in order to obtain the proper distance of the condenser for a particular objective, it is best to put a blood-slide, upon which the corpuscles are in one layer only, on the stage, and project the image on the screen, moving the condenser backward and forward until, when sharply focused, no concentric rings are seen in the disks. The object to be photographed can then be substituted for the blood-slide, and the light will be found to be all that is desired.

When large objects, such as whole insects, are to be photographed under low powers, of small angular aperture, as, for instance, with a three, four or five inch Zentmayer objective, the mode of illumination must be somewhat altered, so as to obtain an evenly illuminated field on the screen. Under such circumstances it is necessary to remove the sub-stage condenser from the microscope and substitute for it a plano-convex lens of some three to four inches focus, placed close to the object. The instrument must then be moved close to the large concentrating lens in the shutter, when it will be found that objects, even as large as three-fourths of an inch in diameter, are evenly illuminated.

APPENDIX.

A short, concise and at the same time comprehensive classification of the more common tumors and neoplasms, in tabular form, such as is given as an appendix to the present volume, will, no doubt, be welcome to the student of pathological histology. Great care has been exercised in its compilation, to introduce all the accepted modern views on the subject, so as to bring it up to the standard of the present time.

The names of tumors and neoplasms heading the different columns are those most commonly used in this country; while under the head of synonyms will be found the terms occasionally employed by continental authors.

The table is divided into four sections: 1, the macroscopic appearances; 2, the microscopic appearances; 3, the clinical features; and 4, a definition; thus giving, at a glance, a clear picture of the life history of the different neoplasms.

The general arrangement is such that the table begins with tumors which are developed in preëxisting, fully formed tissues, and consist mainly in an exaggeration in size, or in a misplacement of the elements naturally

present in the normal tissues, or in other words, homologous tumors—these are essentially non-malignant—and it ends with those tumors which are formed within the fully developed tissues, but consist of more or less developed embryonic elements, which may either belong to the connective tissue series (sarcomata), or to the epithelial series (carcinomata). These are essentially malignant.

Synonyms.

MACROSCOPIC APPEARANCE.

Size, Color and Juice.

Consistence.

MICROSCOPIC APPEARANCE.

Shape and Arrangement of Cells.

Stroma and Vessels.

Degenerations.

CLINICAL CHARACTERISTICS.

Age of Patient.

Glandular Involvement.

Infiltration of Surrounding Parts.

Pain and Rapidity of Growth.

Definition.

CYSTS.	MYXOMA.
Cystic tumor, cystoma, or encysted tumor.	Mucous, gelatinous or colloid tumor; collonema, gelatinous sarcoma and net-celled sarcoma.
Size varies from that of a small seed to that of a man's head and larger; variable in color; juice variable; depends upon contents.	Usually small, but may become very large; if projecting from surfaces may become pedunculated; translucent; greenish, yellowish or yellowish-gray in color; smooth or nodulated in outline; juice clear, viscid, filamentous.
Fluctuating; soft; elastic.	Soft, trembling, gelatiniform.
A cyst is made up of a wall and its contents; the structure varies according to its nature, whether it be a new formation or part of an old tissue; in the latter case it is usually lined with epithelium; the contents vary and are either the natural secretions altered or hemorrhagic and serous effusions.	Angular, stellate and fusiform, with anastomosing prolongations; in young tumors oval and round isolated cells, separated by the intercellular substance. Very abundant; homogeneous, viscid; yielding mucin, or may be fibrous; vessels are not numerous, but with well developed coats and are easily isolated.
The wall may ossify or undergo fatty, mucoid, calcareous degeneration, and may be the seat of secondary cysts, hemorrhagic or otherwise.	May contain hemorrhagic depots and may undergo fibrous or fatty degeneration, and more rarely cartilaginous.
All ages.	Adult age.
None.	Not usual.
None.	Tendency to infiltrate.
Pain depends upon seat and size of cyst; growth is slow.	Pain usually absent; rapid in growth.
A more or less spherical, smooth or lobulated, fluctuating, soft, elastic growth, of indefinite size, being composed of a wall and contents; the former having the character of the tissue from which it sprung, and is usually lined with epithelium; the latter is variable in character, usually being the secretions of a gland somewhat altered, or hemorrhagic or serous effusions.	A greenish-gray, smooth or nodulated mass, of a soft, trembling consistence; usually not larger than an apple; exhibiting under the microscope a highly refractive homogeneous or fibrous stroma, containing stellate and spindle, anastomosing or round and oval cells; the mass being divided into lobules by small bands of connective tissue surrounding the blood vessels.

LIPOMA.	FIBROMA.
Fatty tumor.	Fibrous tumor; connective tissue tumor; desmoid, and issoma.
Usually large; yellow, or pinkish-yellow in color. No juice.	Size varying from that of a small seed to that of a man's head; smooth, round, or lobulated; glistening white, or pinkish, juice not obtainable.
Soft and doughy, with a lobulated surface.	Hard and elastic, creaking under the knife.
Large round cells containing liquid, and sometimes crystallized fat, with the nucleus pushed to the margin; arranged in clusters.	Cells very few in number, spindle-shaped, or stellate in shape, with small, indistinct nucleus.
Bands of fibrous tissue separating the cell clusters into lobules. Numerous fully formed vessels running in the fibrous bands between the lobules.	Stroma composed of white fibrous tissue. The fibres arranged in bundles, which run in different directions through the growth. The vessels are not usually numerous, but well developed, and closely adhere to the fibrous tissue.
The fibrous septa may become exaggerated, or ossified, or calcified; the cells may undergo mucoid degeneration.	May undergo mucoid, fatty, caseous, calcareous and melanotic degeneration. May ossify; may become cavernous, the cavities being filled with blood and in communication with the vessels; if such is the case the new growth is termed an erectile tumor.
All ages.	All ages.
Never occurs.	Does not occur.
Always encapsulated.	Always encapsulated.
Pain depends upon position and size of growth. Growth usually slow.	Pain depends upon position of tumor. Growth usually slow.
A large, soft, elastic, yellow or pinkish mass, lobulated on its surface, presenting under the microscope large round cells filled with liquid, and sometimes crystallized fat, separated into larger or smaller clusters, or lobules, by bands of fibrous tissue.	A round or lobulated, glistening white, or pinkish, hard mass, creaking under the knife, and exhibiting under the microscope bundles of white fibrous tissue running in different directions, with here and there spindle-shaped, or stellate cells, having small and indistinct nuclei.

ANGIOMA.	LYMPHANGIOMA.
Vascular tumor; cavernous tumor; erectile tumor; nævus; arterial vascular tumor.	Lymphatic angioma.
Usually small; red, violet, or bluish purple; no juice except blood.	Small and pale; yields a milky juice.
Soft and elastic; erectile.	Soft, fluctuating, depressible and adherent to the skin.
These growths consist of fully formed capillary blood vessels held together by a small amount of connective tissue, or they consist of a larger amount of fibrous tissue filled with caverns lined with endothelium, communicating with each other and through which the blood circulates freely; or new formation of arteries may predominate.	This growth is made up of a network of dilated lymphatics, held together by a small amount of connective tissue; or it is composed of caverns lined with endothelium, communicating with each other and containing lymph.
Adult life.	Usually congenital.
None.	None.
Always circumscribed.	None.
Pain depends upon seat of growth; of slow growth.	Pain depends upon seat of growth; duration of development uncertain.
A small, soft, but elastic and sometimes erectile mass, of a red, violet or purple color; exhibiting under the microscope a network of vessels held together by a small amount of connective tissue, or of a larger amount of connective tissue containing caverns lined with endothelium and filled with blood.	A small, pale, soft, fluctuating, depressible mass; exhibiting under the microscope a network of dilated lymphatics held together by a small amount of connective tissue, which may surround a system of communicating caverns lined with endothelium and containing lymph.

MYOMA.	NEUROMA.
Muscular tumor; variety, (1) leio-myoma or fibro-myoma; (2) rhabdo-myoma or myoma strio-cellulare, or true myoma.	Nervous tumor.
Usually small; of a pinkish or red color; no juice.	Small; white or yellowish; no juice.
Firm and elastic.	Soft, friable, or partly solid.
Spindle-shaped cells with long, rod-like nuclei; unstriated (leio-myoma) or striated (rhabdo-myoma); lying parallel to each other and forming bands.	Neuromata consist either of ordinary medullated nerve fibres or of ganglionic nerve tissue, and are usually found as small, round tumors on the peripheral nerves, or at the extremities of cut nerves in amputation stumps.
Fibrous connective tissue very abundant; vessels fully developed; usually to be found in the connective tissue.	
May calcify; undergo fatty or mucoid degeneration; contain hemorrhagic effusions or cysts.	May undergo fibrous, myxomatous, glious, and telangiectatic degeneration.
Adult life. Sometimes congenital.	All ages.
None.	Absent.
Usually circumscribed; but may form irregular masses in the mother tissue.	Absent.
Pain depends upon seat of tumor; of slow growth.	Pain usually very severe; rapidity of growth uncertain.
A small, pinkish or reddish, firm and elastic mass, sometimes pedunculated; exhibiting under the microscope large striped or unstriped spindle cells, with large rod-like nuclei; arranged parallel to each other, forming bands; situated in a fibrillated connective tissue stroma.	A small, white, round mass, soft and friable; exhibiting under the microscope the normal appearances of ordinary medullated nerve fibres, or of ganglionic nerve tissue.

CHONDROMA.	OSTEOMA.
Cartilaginous tumor; enchondroma.	Bony tumor; exostosis and endostosis.
Usually small, but may attain a large size; pearly white and glistening; no juice.	Usually small; pinkish or yellowish red in color; no juice.
Hard and elastic; nodular on the surface.	Hard.
Round, oval, lenticular or stellate cells, with a more or less distinct capsule; singly or in groups.	The same as in normal bone.
Hyaline, fibrous, reticulated or mucoid; bands of fibrous tissue separating the growth into lobules: vessels are to be found only in interlobular fibrous tissue.	As in normal bone tissue; eburnated, compact or cancellous; vessels are the same as in normal bone.
May undergo myxomatous, fatty, calcareous and ossific degeneration.	None.
Early life.	Early life.
Very rare; metastasis not unfrequent.	Absent.
Usually encapsulated.	Always circumscribed.
Pain depends upon position and size of growth; rapidity of growth is slow at first; later rapid.	Pain depends upon seat of tumor; of slow development.
A hard, elastic, nodular mass, shiny, pearly white in color; presenting under the microscope large, oval, round, lenticular, or stellate cells, surrounded by a more or less distinct capsule, held singly or in groups in a hyaline, fibrous, or mucoid stroma, which is divided into lobules by bands of fibrous tissue.	A hard, pinkish or yellowish-red mass; exhibiting under the microscope the same structural elements as are seen in normal osseous tissue.

LYMPHAOMA.	ADENOMA.
Lymphatic gland-like, round-celled sarcoma. Lymphadenoma.	Glandular tumor.
Varying in size from a millet seed to that of an adult head; grayish, or yellow-white in color. Juice abundant, clear, containing many granular corpuscles.	Pink, or yellowish-red in color; usually in small nodules; juice clear and scanty.
Soft.	Soft and elastic.
Small granular corpuscles in the reticulated meshes of the stroma.	This tumor being an abnormal development of glandular tissue, is formed by the glandular tubules lined with epithelium, their lumen, however, usually obstructed, and not communicating with the excretory duct of the gland. The vessels which are not numerous are contained in the intra-tubular connective tissue.
Network of stout fibrils, with more or less distinct nuclei at some of the angles of the meshes; vessels numerous, wide, with thin walls.	
May undergo fatty, caseous, mucoid, calcareous, melanotic degeneration, and may contain hemorrhagic depots.	May undergo fatty, caseous, myxomatous, or cystoid degeneration.
Common in young, vigorous subjects.	Youth and adult age.
Very extensive from the first; metastasis frequent.	Secondary glandular involvement in later stages.
Encapsulated.	Usually encapsulated.
Pain caused by pressure of tumor upon neighboring nerves only; duration of growth uncertain, sometimes rapid.	Pain not severe; growth slow.
A grayish, or yellow-white, soft mass, smooth, or nodulated, varying in size from that of a millet seed to that of an adult head, and yielding an abundant juice, rich in cellular elements. On microscopic examination it presents small, round, granular corpuscles, contained in the meshes of a delicate reticulated, fibrillated stroma, which shows more or less distinct nuclei at the angles of some of its meshes.	A soft, elastic, pinkish mass, exhibiting under the microscope abnormal development of glandular tissue, the ducts of which are, however, not communicating with the excretory duct of the gland, and are usually obstructed by cell debris and mucoid material.

PAPILLOMA.	SYPHILOMA.
Warty growth; condyloma; cauliflower excrescence; villous tumor.	Syphilitic tumor; gummy tumor.
Varying from the size of a pea to that of a walnut; pinkish or whitish; no juice except blood.	Varying in size from that of a hemp seed to that of a small orage; yellowish-white, or pinkish in color; globular, smooth, more or less circumscribed; no juice.
Hard, often horny; or soft, according to situation.	Moderately firm and elastic.
The new growth, consisting of an undue development of the normal papillæ of the skin or mucous membrane, exhibits all the features of the normal structure, but is frequently much more vascular.	Small, round, granular cells, massed and compressed together at the circumference of the nodule; depot of cell debris in the centre. Stroma consists of incompletely fibrillated tissue; vessels few, but well developed on the circumference; become occluded toward the centre and disappear.
Ulceration or hemorrhage.	Usually undergoes caseous or fatty degeneration, but may suppurate or form cicatricial tissue.
All ages.	All ages.
Absent.	Usual in later stages of disease.
Never infiltrating.	Surrounding parts not infiltrated.
Pain usually absent; rapidity of growth variable.	Pain depends upon location; usually absent; of slow growth.
A small, pinkish or whitish mass, of irregular outline, situated on the skin or mucous membrane; exhibiting under the microscope the normal structure of skin or mucous membrane. The growths are usually highly vascular, and are apt to ulcerate.	A globular, smooth, elastic, pinkish or yellowish-white nodule, varying in size from that of a hemp seed to that of a small orange; presenting under the microscope a central depot of cell debris, surrounded by small, round, granular cells, contained in more or less imperfectly fibrillated stroma.

TUBERCLE.	GLIOMA.
Miliary tubercle; gray granulation.	Glious tumor; neuroglioma.
Small masses, up to the size of a millet seed; gray or yellowish, semi-transparent; juice cannot be obtained.	Usually small, but may become large from hemorrhagic effusions; pinkish or grayish white; clear juice, containing granular corpuscles.
Resisting under pressure.	Soft, and easily crushed; irrregular in outline.
Free nuclei, granular small cells; larger cells, with many nuclei massed together; surrounded by adenoid tissue.	Small granular corpuscles, resembling the white blood corpuscles; occasionally larger, and rarely fusiform; massed together.
Absent, or a scanty, very delicate network of adenoid tissue; vessels are not perceptible.	Soft, scanty, amorphous; occasionally fibrillated; vessels of mother tissue in greater part maintained.
Usually undergoes caseous degeneration, but may also become the seat of fatty, mucoid and calcareous degeneration.	May undergo fatty caseous, calcareous, melanotic, and mucoid degeneration.
May occur at all ages.	Infancy and early youth.
Usually in later stages.	Occurs in later stages only.
Never encapsulated.	Never encapsulated.
Pain depending upon position of tubercles; duration of growth cannot be determined.	Little, if any pain; of rapid growth.
A small, gray, semi-translucent or yellowish opaque nodule, up to the size of a millet seed; resisting under pressure; presenting under the microscope free nuclei, small, round, granular and large, many—nucleated cells, with no visible stroma, but the mass of cells surrounded by adenoid tissue.	A soft, grayish, or pinkish-white mass; occurring primarily in the orbit and brain; composed of small, round, granular cells; with one or two bright spots in the centre; arranged in masses, in a delicate amorphous intercellular substance.

ROUND-CELLED SARCOMA.	SPINDLE-CELLED SARCOMA.
Sarcoma globo-cellulare simplex; embryoplastic tumor; granulation tumor; encephaloid sarcoma; medullary sarcoma; alveolar sarcoma; lymphadenoid sarcoma.	Recurrent fibroid; fibro-plastic tumor; fibro-nucleated tumor; fibrous sarcoma; plasmoma, and fasciculated sarcoma.
Yellowish, or pinkish, or brain-like. May become very bulky. Scanty, clear juice, containing but few cells when fresh. Abundant, creamy, containing many cells after twenty-four hours.	Usually small, but may attain large dimensions; of grayish-white color. The juice is scanty, containing few cells.
Soft, elastic, homogeneous.	Hard and firm.
Small round or large oval cells with large nucleolated nuclei; the protoplasm of the cells small in amount and the cells in close contact with each other.	Large or small fusiform or spindle cells, with oval nuclei and tapering ends, arranged parallel to each other in strands, or trabeculæ, which run in different directions.
Soft, scanty, and amorphous; sometimes fibrillated, sometimes alveolar, sometimes lymphatic gland-like; capillaries mere channels between the cells.	Scanty and homogeneous, or fibrillated. Vessels are numerous, but without walls.
May become ossified or calcified. The cells may undergo fatty, mucous, caseous or melanotic degeneration.	May undergo melanotic, fatty, cystoid, myxomatous, caseous, calcareous, ossific and telangiectatic degenerations.
All ages.	All ages.
Seldom met with. Metastasis not unfrequent.	Not usual, but may occur. Metastasis frequent.
Common.	Very common.
Pain usually dull, and not frequent. Growth not usually rapid.	Pain dull and persistent in most cases. Usually of rapid growth.
A soft and elastic mass, usually very vascular, of a pinkish or yellowish-red color, yielding when fresh a scanty, clear juice, and presenting on microscopic examination small round or larger oval cells, with large nucleolated nuclei embedded in a scanty, soft and amorphous, but sometimes fibrillated stroma, with the walls of the vessels but slightly developed and the capillaries mere channels between the cells.	A white or pinkish, hard and firm mass, yielding a scanty juice, which presents under the microscope small or large spindle cells, with oval nuclei. The cells being parallel with each other, and arranged in bundles which run in different directions through the growth, giving the appearance of oval or round cells, when cut transversely.

GIANT-CELLED SARCOMA.	EPITHELIOMA.
Myeloid tumor; tumeur a myiloplaxes; myeloplastic tumor.	Epithelial carcinoma; epithelial cancer; epidermal cancer; cancroid; squamous epithelioma.
From hazelnut to adult head; maroon, or reddish brown, or yellowish white; dotted with crimson; the juice is scanty.	Extremely variable in size and color; juice scanty, thick and stringy.
Moderately firm.	Hard, resisting; forming ulcers with raised edges, in later stages.
Large, polymorphous, and odd multinucleated cells, separated by granular intercellular substance.	Large, squamous, epithelial cells, arranged in compact masses; with a tendency to a concentric arrangement; forming the so-called "nests," or "pearls."
Stroma of spindle and round cells; vessels rather numerous; with thin, if any walls.	The cell masses are separated by the connective tissue, which is infiltrated by granulation cells; numerous vessels are found in the connective tissue.
May undergo fatty, cheesy, mucoid, cystoid, calcareous, osseous, cartilaginous, fibrous, and telangiectatic degeneration.	The cells may undergo a fatty, or mucoid degeneration; the growth may become calcified, or even ossified.
Usually before thirtieth year.	Advanced life.
Occasional.	Common in later stages; but depending upon the position of the primary growth.
Encapsulated; but occasionally infiltrating.	Infiltrating from the first.
No pain, as a rule, and of rather rapid growth.	Pain extremely severe in later stages; usually of slow growth.
A moderately firm, reddish brown, encapsuled mass; which presents, under the microscope, multi-nucleated, ovoid and queerly-shaped cells, in a stroma of spindle and round cells.	A hard mass of variable size and color; usually ulcerating on the surface; yielding a scant, thick juice, containing epithelial cells; exhibiting, under the microscope, compact masses of large, squamous, epithelial cells, with a tendency to a concentric arrangement, and the formation of "nests," or "pearls." the cell masses being separated by the subcutaneous or submucous connective tissue, in a state of inflammatory infiltration.

CYLINDER-CELLED EPITHELIOMA.	SCIRRHUS.
Columnar epithelioma; villous cancer.	Hard carcinoma; simple carcinoma; connective tissue cancer; fibrous cancer; chronic cancer; tubular cancer.
Variable in size; yellowish or pinkish white in color; abundant milky juice, containing many cells.	Varying in size from that of a pea to that of a fist; milky juice, containing many cells.
Soft and easily crushed.	Dense and hard.
Columnar epithelial cells lining the inner surface of tubules.	Epithelioid cells of various shapes, with large, oval, nucleolated nuclei, disorderly grouped together, floating loosely in a clear liquid, contained in elongated alveoli of the stroma.
Fibrillated connective tissue, forming tubes or cylinders; fully formed vessels running in the fibrillated stroma between the cylinders.	A more or less fibrillated, dense tissue, containing alveoli, in which the cells are grouped together, and into which open lymphatic vessels; numerous blood vessels contained in the stroma.
May undergo fatty and mucoid degeneration.	The tumor may become ossified, cartilaginous or calcified; the cells usually rapidly undergo fatty degeneration.
Adult life.	Later adult age.
Usual in later stages. Metastasis frequent.	Very common; metastasis frequent.
Always infiltrating.	Always infiltrating.
Pain severe; of slow growth.	Usually severe, lancinating and intermitting pain; of slow growth.
A soft, reddish mass, springing primarily from mucous membranes; exhibiting under the microscope tubules of connective tissue, lined on their inner surface with columnar epithelial cells.	A hard, white nodule, yielding an abundant milky juice, containing many cells; exhibiting under the microscope a more or less fibrillated, dense, cavernous framework, whose alveoli are filled with loose cells of an epithelial type, grouped together disorderly, bathed in a clear fluid, and having no visible intercellular material.

ENCEPHALOID CARCINOMA.	COLLOID CARCINOMA.
Medullary, soft, acute, acinous carcinoma.	Alveolar cancer; gelatinous cancer.
Variable in size; yellowish-white, or pinkish; abundant, creamy juice, containing many cells.	May attain an enormous size; translucent amber color; gelatinous juice, containing few cells.
Frequently lobulated; soft; brain-like.	Jelly-like; trembling.
Epithelial cells of various shapes, with large nucleolated nuclei.	Large ovoid cells, with large nucleolated nuclei; these are surrounded by concentric rings, grouped irregularly in the middle of the alveoli of the stroma.
Slender trabeculæ of fibrous tissue, forming a large meshed network; vessels abundant in the stroma.	A network of fibrillated tissue, forming regular round alveoli, of various sizes, visible to the naked eye, and filled with a gelatinous mass, holding the epithelioid cells in the centre; vessels not numerous, and small, running in inter-alveolar tissue.
The cells rapidly undergo fatty degeneration; rupture of vessels and hemorrhage frequently follow the fatty or mucoid degeneration of the stroma.	May undergo fatty, and mucoid degeneration.
Advanced adult life.	Adult life.
Common. Metastasis very frequent.	Usually absent.
Infiltrating from the first.	Always infiltrating.
Severe, intermitting pain; of rapid growth.	Pain slight; of very rapid growth.
A lobulated, pinkish, or yellowish, soft, brain-like mass, yielding an abundant creamy juice, and exhibiting under the microscope epithelioid cells with large nucleolated nuclei, floating loosely in the large ovoid meshes, or alveoli of a fibrous stroma.	A large, trembling mass, of an amber color; yielding a gelatinous, stringy juice, containing few cells; exhibiting under the microscope, round alveoli, formed by a network of fibrillated connective tissue, containing in their lumina a tenacious substance, and in the centre a few ovoid large cells, of an epithelial type, with large oval nucleolated nuclei.

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